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THE "MINNESOTA CODE" FOR ECG CLASSIFICATION ADAPTATION TO CR LEADS AND MODIFICATION OF THE CODE FOR ECGs RECORDED DURING AND AFTER EXERCISE

By

The Scandinavian Committee on ECG Classification

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It has been clearly demonstrated that conventional clinical methods of ECG interpretation carry a large inter- and intraindividual variability (1, 12 13). It has also been shown that strict adherence to a set of welldefined ECG-criteria *will reduce observer errors* (7 8). The ECG classification system proposed by Blackburn et al (8), the "Minnesota Code" has been extensively used in epidemiological studies of cardiovascular disease for more than five years and it has won wider acceptance than any other classification method.

A Scandinavian Technical Committee on ECG Classification was formed in 1963 sponsored by and including representatives of the national societies of Cardiology and Clinical Physiology in Denmark Finland Norway and Sweden. The committee met in Helsinki Finland in 1965, and in Linköping, Sweden in 1966. It was generally felt among its members that the "Minnesota Code" represented the best available system for objective classification of ECG findings but that applications of the code in its present form were limited primarily to epidemiological studies for which it was intended. The code is based on a 12 lead ECG including leads I II III aVR aVL aVF, and V_1 through V_6 while CR leads are standard in many Scandinavian hospitals (and used to some extent in Great Britain and Russia). It was also pointed out that data from recent studies have indicated that the original classification of ST *changes is not sufficiently detailed* for optimal characterization of the findings to exercise ECG response.

The Committee concluded that a series of additions to the original code and an adaptation to CR leads were desirable to extend its usefulness within the countries represented by the Committee members. The modifications of the code fall mainly within two areas—those directly related to the use of CR leads i e changes in amplitude criteria and those concerning the classification of ECG changes during and after exercise. The Committee also made recommendations on a protocol to be used in exercise testing.

A revised edition of the original "Minnesota Code" to be published in a WHO manual by Rose and Blackburn (27) on cardiovascular population studies was made available to the committee during the final stage of preparation of this report, and the Scandinavian code has been coordinated with the new WHO edition.

One of the factors in the preferential use of CR leads in many Scandinavian hospitals is the current methodology for exercise testing which calls for recording of multiple chest leads during exercise This is technically difficult to accomplish with standard V leads but can be done easily with bipolar CH (H for head, or forehead) leads Holmgren and Strandell (18) have demonstrated that differences between CR and CH leads are generally very small and that the leads are interchangeable for clinical applications CR leads are usually recorded before and after exercise and to *avoid motion artefacts CH leads during* exercise The common usage of CR leads includes position C_7 and excludes position C_4 It is felt that CR_4 rarely reveals any information that is not present in lead CR_6 while lead CR_7 provides an optimal lead axis for display of ECG changes originating in the lateral wall of the left ventricle Amplitudes in lead V_7 are generally small but this disadvantage does not apply to CR_7 Evans (14) has advocated the use of CR_7 for similar reason

Several comparisons between CR and V leads have been reported in the literature (6 9 16 21 However there is to our knowledge no study aimed at the translation of a complete set of criteria from V leads to CR leads Two members of the committee Drs Irma Åstrand and Gunnar Blomqvist made available the results of a study comparing CR and V leads in a group of 50 male and female normal subjects of ages 20 to 69, 40 patients with ST-T segment and ST-J changes and 40 patients with myocardial infarction Details on material and methods are presented in the Appendix, Part II, where the results are given in terms of amplitudes and durations

Results of significance with regard to the use of the "Minnesota Code" are discussed below Conversion factors have been based on mean values Skewness of certain amplitude distributions has been disregarded Three-digit decimal coded numbers in the text refer to various items in the WHO version of *the Minnesota Code (27) A complete* modified code for use with CR leads is given in the Appendix, Part I

Intervals Measurements of PR, Q QRS and QT durations in CR leads gave generally somewhat higher values than corresponding measurements in V leads This was true in all groups studied Discrepancies with regard to PR and QT may be disregarded as these intervals according to the code, are measured in frontal plane leads I, II, and III Differences in Q wave and QRS durations were small in relation to the accuracy of the measurements and would affect the classification only in occasional cases

1 Q and QS items The classification of Q waves in the horizontal plane leads, measurements of Q/R amplitude ratios and Q wave durations presence of QS complexes in V and CR leads in the group of 40 patients with myocardial

infarction resulted in identical classifications of all cases without any modification of the original code. The series included cases covering all major categories of the code.

Q/R ratios were measured in chest positions C_1, C_2, C_4 and C_7. Differences between CR and V leads were small and inconsistent in the infarct material. The following mean values were found in the infarct cases:
CR_2—0.13 and V_2—0.14, CR_4—0.29 and V_4—0.28, CR_5—0.23 and V_5—0.26, CR_7—0.19 and V_7—0.15. Q/R ratio differences were also insignificant in the normal group and the group with ST-T changes. No Q waves were present in CR_2—V_2 in these groups.

Item 1.2.8 specifies "decreasing absolute R amplitude and smallest R wave amplitude 0.2 mV or less V_2 to V_3, V_3 to V_4, etc., i.e. the R wave in V_3 or V_4 or beyond should be no larger than 0.2 mV. It is apparent from the tables in the appendix that the average R wave amplitude in leads CR_2 and CR_4 is approximately 20 per cent larger than in lead V_2 and V_4. Thus the 0.2 mV criterion in item 1.2.8 should be increased to 0.25 mV (measurements are made to the nearest 0.05 mV or 0.5 mm).

No additional adjustments of category Q+QS criteria are required.

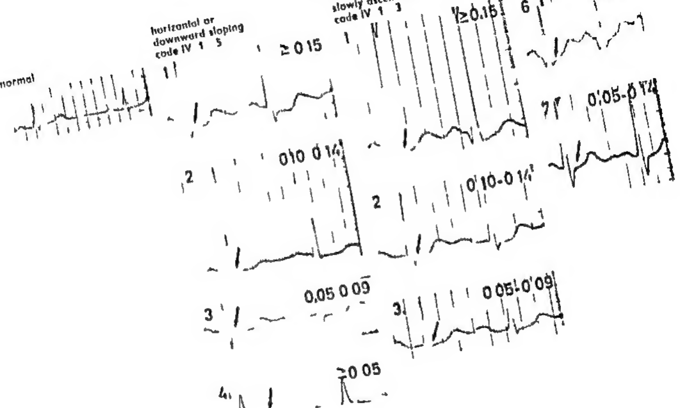
2. *Axis deviation.* The classification is based only on frontal plane leads I, II, and III and is unaffected by CR-V differences.

3. *Tall R waves.* R wave amplitudes equalling or exceeding 2.6 mV in leads V_5 or V_6 are coded as 3.1. However R waves in CR_5 were on the average 24 per cent taller than in V_5 in the normal group. Corresponding figures in the groups with ST-T changes and with myocardial infarction were 23 and 29 per cent. Similar comparisons were carried out for CR_7—V_7, and gave differences of 39, 56 and 33 per cent. The R wave was taller in CR_5/V_5 than in CR_7/V_7 in all subjects of the normal group. The same was also true in the group with ST changes and the use of CR_7 rather than CR_5 will apparently not influence the classification.

A single modification is thus required in item 3.1: "R wave amplitude in $CR_{5,7}$ 3.3 mV or more" replaces "R wave amplitude in $V_{5,6}$ of 2.6 mV or more".

The original criterion for tall R waves of the right ventricular type specifies an R wave amplitude of 0.5 mV or more and an R/S ratio of 1.0 or more in lead V_1. Analysis of the group of normal subjects showed a mean R amplitude in CR_1 20 per cent above the mean in V_1. Corresponding figures for the groups with ST changes and the infarct material was 10 per cent over V lead values. The R/S ratio in the normal group was 0.96 in CR_1 and 0.46 in V_1. Mean values in the group with ST changes were 0.98 and 0.68. These data suggest that an R wave amplitude criterion of 0.6 mV or greater and an R/S ratio of 1.5 or greater should be used for CR_1.

4. *ST-J and ST segment changes.* Limits of 0.05 and 0.1 mV are used in the classification of ST-J findings in V leads. Most of the patients with ST changes in this study group showed a maximal ST depression in leads CR_4/V_4 and CR_5/V_5. Negative ST amplitudes were on the average 25 per cent larger in the two CR leads, i.e. the same relative



ST segment
normal
horizontal or
downward sloping
code IV 1 5
≥ 0 15
straight and
slowly ascending
code IV 1 3
≥ 0.15
upward sloping
code IV 6–7
≥ 0 15
6
7
0,05-0 14
2
0 10 0 14
2
0 10-0 14
3
0,05 0 09
3
0 05-0 09
4
≥ 0 05

Classification of ECG changes during and after exercise

ST changes The original code (8) did not consider the shape of the ST segment. An isolated J depression of 0.1 mV or more was referred to the same group as a horizontal or downward sloping depression of the same magnitude. A modification was suggested by Blomqvist and Åstrand (11) and has subsequently been used in several studies (5, 17, 20, 22, 24). The modified "Minnesota Code" (27) differentiates between junctional and horizontal or downward sloping depression. However, a large ST-J depression with a straight, slowly ascending but not strictly horizontal ST segment not reaching baseline before T wave is not infrequently seen in patients with unquestionable angina pectoris. This is particularly common during and immediately after exercise with marked tachycardia. The great emphasis on the requirement of a strictly horizontal or downward sloping ST segment is mainly based on Mattingly's follow up studies (23), but it should be noted that only ECGs recorded *after* exercise at a moderately heavy work load (Master's double step test) were included in his analyses. Preliminary results from a Finnish population study (25) suggest that ST-J depressions with straight slowly ascending ST segments probably have the same prognostic significance as ST-J depressions with a horizontal or downward sloping ST segments (Fig. 1). The committee is aware of the fact that "straight slowly ascending" constitutes a less stringent definition than "horizontal" and may introduce a larger inter- and intra-observer variability. It recognized the need for further follow-up studies to confirm preliminary findings in the Finnish material. However, the decision to include straight but not strictly horizontal ST depression had strong qualitative support in the large clinical experience with multiple load exercise testing accumulated by members of the group.

T wave changes It was felt by the Committee that it frequently is desirable to record also minor T wave changes. A new subgroup is added to the fifth category (5-4), including T wave amplitudes less than 0.20 mV.

A negative T wave in the ECG recorded at rest often becomes a low positive one during exercise (cf. 2).

Ectopic beats Careful studies have failed to reveal any relationship between the occurrence of ectopic beats (supraventricular and ventricular) and ST depressions during and after exercise (4, 29). A detailed classification of ectopic beats may not be of primary interest in the study of coronary disease but is potentially useful in other conditions and has been added to the code, category 10.

Heart rate and work load Electrocardiographic changes during and after exercise are quantitatively related to relative work load and heart rate (3, 10, 19, 29). The heart rate and the work load should therefore always be given whenever ECG is coded. It is recommended that

e g a punch card sortee Muluple codable items within the same category (i e 8 9 10) are therefore identified only as the presence of a combination of two or more items Use of more sophisticated data processing equipment makes the requirement that items be mutually exclu sive less stringent Items recording combina tions may be disregarded and each finding within categories 8 9 and 10 may be coded separately It should be noted that items within each category 1 to 7 have been made mutually exclusive generally by letting the lowest second digit number represent the most significant deviation and not reporting less significant find ings The reader is referred to the new WHO edition of the "Minnesota Code (27) for V lead criteria and a more elaborate set of proce dural rules

The Scandinavian code departs from the WHO edition with respect to coding ST and T items It may also be used with V leads ST and T amplitude criteria for leads I II III aVL and aVF should then be applied for chest leads V_1 through V_6

The "Minnesota code" for ECG classification
Adaptation to CR (CH) leads and modification of the code for ECGs recorded during and after exercise

0 0 0 Blank — no ECG available
1 0 0 No herein reportable ECG items

Q and QS patterns

(Do not code in presence of ventricular conduction defects 6 4 or 7 1)

1 1 1 Q/R amplitude ratio 1/3 or more plus Q duration 0 03 sec or more in any of leads *I II* $CR_{1\ 3\ 4\ 5\ 6\ 7}$

1 1 2 Q duration 0 04 sec or more in any of leads *I II* $CR_{1\ 2\ 3\ 4\ 5\ 6\ 7}$

1 1 3 Q duration 0 04 sec or more *plus* R amplitude of 3 mm or more in lead *aVL*

1 1 4 Q duration 0 05 sec or more in lead *III plus* any Q wave of at least 1 0 mm amplitude in *aVF*

1 1 5 Q duration 0 05 sec or more in lead *aVF*

1 1 6 QS pattern when R wave is present in adjacent lead to the right on the chest in any of leads $CR_{1\ 3\ 4\ 5\ 6\ 7}$

1 1 7 QS pattern in all of leads CR_1 through $CR_{3\ 4}$ or

1 2 1 Q/R amplitude ratio 1/3 or more *plus* Q duration at least 0 02 sec and less than 0 03 sec in any of leads *I II* $CR_{1\ 3\ 4\ 5\ \ 7}$

1 2 2 Q duration at least 0 03 sec and less than 0 04 sec in any of leads *I II* $CR_{1\ 3\ 4\ 5\ 6\ 7}$

1 2 3 QS pattern in lead *II*

1 2 4 Q duration of at least 0 04 sec and less than 0 05 sec in lead *III plus* any Q wave of at least 1 0 mm amplitude in *aVF*

1 2 5 Q duration at least 0 04 sec and less than 0 05 sec in lead *aVF*

1 2 6 Q amplitude of 5 mm or more in either of leads *III aVF*

1 2 7 QS pattern in all of leads CR_1 through *CR*

1 2 8 R amplitude decreasing to 2 5 mm or less and absence of codes 3 2 7 2 or 7 3 between any of leads *CR and* CR_4 CR_3 *and CR* CR_4 *and* CR_5 CR_5 *and* CR_6 *or* CR_6 *and* CR_7

1 3 1 Q/R amplitude ratio at least 1/5 and less than 1/3 *plus* Q duration of at least 0 02 sec and less than 0 03 sec in any of leads *I II* $CR_{1\ 2\ \ 3\ 4}$

1 3 2 QS pattern in absence of code 3 1 in each of leads *CR and* CR_4

1 3 3 Q duration of at least 0 03 sec and less than 0 04 sec *plus* R amplitude of 3 mm or more in lead *aVL*

1 3 4 Q duration of at least 0 03 sec and less than 0 04 sec in lead *III plus* any Q wave of at least 1 0 mm amplitude in lead *aVF*

1 3 5 Q duration of at least 0 03 sec and less than 0 04 sec in lead *aVF*

1 3 6 QS pattern in each of leads *III and aVF*

QRS Axis deviation

(Do not code in presence of low voltage QRS code 9 1 or ventricular conduction defect 6 4 7 1 2 4)

2 1 Left

QRS Axis from —30 through —90 in leads *I II III* (The algebraic sum of major positive and major negative QRS waves must be zero or positive in *I* negative in *III* and zero or negative in *II*)

2 2 Right

QRS Axis from +120 through −150 in leads *I II III* (The algebraic sum of major positive and major negative QRS waves must be negative in *I* and zero or positive in *III* and in *I* must be one half or more of that in *III*)

2 3 Right

(optional code when 2 2 is not present)

QRS axis *from* +90 through +119 *in leads I II III (The algebraic sum of major positive* and major *negative* QRS *waves must* be zero or negative in *I* and positive in *II* and *III*)

2 4 Extreme axis deviation (usually S_1 S_2 S_3 pattern)

QRS axis from −90 *through* −149 in leads *I II and III* (The algebraic sum of major positive and major negative QRS waves must be negative in each of leads *I II and III*)

2 5 Indeterminate axis

QRS axis approximately 90 from the frontal plane (The algebraic sum of major positive and major negative QRS waves is zero in each of leads *I II and III* or the information from these three leads is incongruous)

High amplitude R waves

(Do not code in presence of ventricular conduction defect 6 4 7 1 2 4)

3 1 *Left*

R *amplitude greater than 33 mm in either of leads $CR_{4\ or\ 5}$*

R amplitude greater than 20 mm in any of leads *I II III aVF*

R amplitude greater than *12 mm* in lead *aVL*

3 2 Right

R amplitude equal *to or* greater than 6 0 *mm* and R amplitude equal to or greater than S amplitude in lead CR_1 when a decreasing R S amplitude ratio occurs somewhere to the left of CR_1 on the chest (Includes code 3 3 which meets the above criteria)

3 3 Left (optional code when 3 1 is not present)

R amplitude greater than 15 mm but less than 20 *mm* in lead *I or*

R amplitude in $CR_{4\ or\ 5}$ *plus* S amplitude in CR_1 greater than 40 *mm*

ST depressions

(Do not code in presence of ventricular conduction defects 6 4 7 1 2 4)

4 1	ST J *depression of 1 5 mm or more* or *1 0 mm or more* *and* ST segment straight and slowly ascending *horizontal* or downward sloping	$CR_{\ }$ I II aVL aVF
4 2	ST J depression of 1 0 −1 4 mm *and* ST segment straight and slowly ascending horizontal or downward sloping	$CR_{\ }$
4 3	ST J depression of 0 5 0 9 mm *and* ST segment straight and slowly ascending horizontal or downward sloping	CR_1 I II aVL aVF
4 4	No ST J depression as much as 0 5 mm *but* ST segment downward sloping and reaching 0 5 mm or more below P R baseline	CR I II aVL aVF
4 5	No ST J depression as much as 0 5 mm *but* ST segment horizontal or downward sloping but reaching less than 0 5 mm below P R baseline	$CR_{\ }$ I II aVL aVF
4 6	Isolated ST J depression of 1 5 mm or more or 1 0 mm or *more* ST segment upward sloping	$CR_{\ }$ I II aVL aVF

4 7	Isolated ST J depression of 0.5—1.4 mm or more	CR_{1-7}
	or 0.5—0.9 mm or more	I, II, aVL, aVF
	ST segment upward sloping	

T wave items

	(Do not code in presence of ventricular conduction defects 6 4, 7 1, 7 4)	
5 1	T amplitude = minus 5 mm or more	I, II
	when R amplitude = 5 mm or more	aVL
	when QRS mainly upright	aVF
	T wave amplitude = minus 6.5 mm or more	CR_{2-7}
5 2	T amplitude = minus 1 to 5 mm (positive negative or negative positive type) when R amplitude = 5 mm or more	I, II
		aVL
	when QRS mainly upright	aVF
	T wave amplitude = minus 1.5 to 6.4 mm	CR_{2-7}
5 3	T amplitude zero (flat) or negative or diphasic (negative positive type)	
	with less than 1.0 mm negative phase in	I, II
	less than 1.5 mm in	CR_{4-7}
	when R amplitude = 5 mm or more	aVL
	not coded in	aVF
5 4	T wave low (less than 2 mm)	I, II, CR_{2-7}
	if R = 5 mm or more	aVL
	if QRS mainly upright	aVF

A V conduction defect

6 1 Complete (third degree) A V block (permanent or intermittent) in any lead

6 2 Partial (second degree) A V block in any lead (2:1, 3:1 block, Wenckebach etc.)

6 3 P R (P Q) interval 0.22 sec or more in any of leads *I, II, III, aVL, aVF*

6 4 Wolff Parkinson White syndrome
P R (P Q) interval less than 0.12 sec *plus* QRS duration 0.12 sec or more *plus* R peak duration 0.06 sec or more coexisting in the same beats of any of leads *I, II, aVL,* $CR_{4, 5, 6 \text{ or } 7}$

6 5 Short P R (P Q) interval
P R (P Q) interval less than 0.12 sec in any two of leads *I, II, III, aVL, aVF*

Ventricular conduction defect

7 1 Complete left bundle branch block (in absence of 6 4)
QRS duration 0.12 sec or more in any of leads *I, II, III, aVL, aVF* and R peak duration 0.06 sec or more in any of leads *I, II, aVL,* $CR_{5, 6 \text{ or } 7}$

7 2 Complete right bundle branch block (in absence of 6 4)
QRS duration 0.12 sec or more in any of leads *I, II, III, aVL, aVF plus* R prime greater than R or R peak duration 0.06 sec or more in leads *CR* or CR_2

7 3 Incomplete right bundle branch block
QRS duration less than 0.12 sec in each of leads *I, II, III, aVL, aVF* and R prime greater than R in either of leads CR , (Report as 3.2 if those criteria met.)

7 4 Intraventricular block (in absence of 6 4, 7 1 or 7 2)
QRS duration 0.12 sec or more in any of leads *I, II, III, aVL, aVF*

7 5 R R prime not meeting criteria of 7 2 or 7 3 in either of leads CR_1 or *CR*

7 6 Incomplete left bundle branch block
QRS duration at least 0.10 sec and less than 0.12 sec in the absence of Q waves in each of leads *I, aVL and* $CR_{5, 6 \text{ or } 7}$

Arrhytmias

8 1 Any combination of arrhytmias below
8 2 Ventricular tachycardia (over 100/min)
8 3 Atrial fibrillation or flutter
8 4 Supra ventricular tachycardia (over 100 min)
8 5 *Ventricular (idioventricular) rhythm (up to 100/min)*
8 6 A V nodal rhythm (up to 100/min) Defined as a negative P wave in lead *aV F plus a* P R interval of 0 12 sec or less in any two of leads *I II III aV L aV F*
8 7 Sinus tachycardia (over 100/min)
8 8 Sinus bradycardia (under 50/min)
8 9 Arrhytmias not mentioned above

Miscellaneous items (coded at rest only)

Amplitudes within parentheses apply to leads I II III aV L, aV F
9 1 *Any combination of items below (to be coded only when defined or else not listed in totals)*
9 2 Low QRS amplitude
QRS peak to-peak amplitude less than 5 mm in each of leads *I II III* or QRS peak to-peak amplitude less than 13 mm in each of leads $CR_{1\ 2\ 3\ 4\ 5\ 6\ 7}$
9 3 ST J elevation of 2 mm or more — $CR_{1\ 4}$
ST J elevation of 1 5 mm or more — $CR_{5\ 7}$
ST J elevation of 1 0 mm or more — I II III aV L aVF
(Do not code in the presence of codes 6 4 7 1 7 2 or 7 4)
9 4 P wave amplitude of 2 5 mm or more of leads *II III or aV F or 3 5 mm* in $CR_{1\ 7}$
9 5 QRS transition zone at lead CR_4 or to the left of CR_4 on the chest
9 6 T wave amplitude greater than 15 mm (12 mm) in any of leads *I II III aV L, aV F* $CR_{1\ 2\ 3\ 4\ 5\ 6\ 7}$
9 7 Findings questionable due to wandering baseline noise or other technical defect in the record

Ectopic Beats

10 1 Frequent (more than 5 out of 20) multifocal ventricular premature beats
10 2 Frequent (more than 5 out of 20) unifocal ventricular premature beats
10 3 Multifocal (2 to 4 out of 20) ventricular premature beats
10 4 Unifocal (2 to 4 out of 20) ventricular premature beats
10 5 *Occasional ventricular premature* beats
10 6 Frequent (more than 5 out of 20) supraventricular (including nodal) premature beats
10 7 Supraventricular (including nodal) premature beats (2 in 4 out of 20)
10 8 Occasional supraventricular (including nodal) premature beats
10 9 Combination of any 1—4 and any of 8 and 7

Medication

11 1 *Digitalis and other cardiac glycosides*
11 2 Quinidine or procaine amide
11 3 Propranolol or other adrenergic beta receptor blocking drug
11 4 Psychopharmaca
11 5 Diuretics
11 6 Combinations 1 and 2 1 and 3 1 and 4 or 1 and 5
11 7 Combinations 2 and 3 2 and 4 2 and 5

11 8 Combinations 3 and 4 3 and 5
11 9 Combinations 4 and 5

Most marked symptom during and/or after exercise (orthostatic test)

12 1 Typical angina pectoris (Rose s criteria 26)
12 2 Other chest discomfort
12 3 Dyspnea
12 4 Claudicatio
12 5 Exhaustion fatigue leg discomfort
12 6 Dizziness vertigo

Heart rate

13 Heart rate 135 will be coded as 13 1
14 14 3 15 5
15

Work load

16 Work load 300 kpm/min* will be coded as 16 [illegible] 17 3
17 18 0 19 0 1200 as 16 1 17 2 18 0 19 0 2 70 V_O L/min will be coded as 16 [illegible] 17 2
18 18 7 [illegible] [illegible]
19

* 300 kpm/min=approx 50 watts

PART II

Amplitudes and durations measured in CR and V leads

Material and methods The material included 50 unselected normal subjects (normal according to clinical and laboratory findings) sampled from an extensive population study, 10 from each decade 20—69 years, and an equal number of males and females In addition 40 consecutive cases demonstrating ST and ST J changes and 40 consecutive cases with a clinical and electrocardiographic diagnosis of myocardial infarction were drawn from the files of the Laboratory of Clinical Physiology, Serafimerlasarettet Stockholm An ECG recorded approximately 2 weeks after onset of clinical symptoms was selected for analysis in the infarct cases All ECG s were recorded at 50 mm/sec on a four channel inkwriter (jet) with frequency response curve flat beyond 600 cps (Mingograf 42 B, Elema—Schonander Stockholm) All tracings included the conventional six frontal plane leads and V and CR leads from chest positions 1, 2 4 5 and 7 The PR segment at the beginning of QRS was used as a reference in all amplitude measurements Amplitudes were measured to the nearest 0 5 mm or 0 05 mV and durations also to the nearest 0 5 mm or 10 msec

TABLE 1 PR interval (c sec) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parentheses and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_5 V_5	CR_7 V_7
N_1	142 138	140 136	146 140	146 140	144 144
	(3)±08	(3)±08	(4)±24	(4)±14	(0)±14
N_2	144 148	144 146	156 152	142 138	154 150
	(3)±08	(1)±02	(3)±16	(3)±10	(3)±08
N_3	156 152	154±152	164 164	164 154	168 154
	(3)±08	(1)±04	(0)±16	(6)±14	(8)±18
N_4	174 166	174 168	182 168	182 166	180 162
	(5)±20	(3)−10	(8)−10	(9)±12	(10)−14
N_5	154 152	162 150	168 160	166 160	170 156
	(1)±10	(7)±10	(5)±18	(4)±08	(8)±10
N_{1-5}	154 152	154 150	164 158	160 152	164 154
	(1)±12	(3)+08	(4)−14	(5)+12	(F)−14

TABLE 2 Q duration (c sec) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3 40—49 N_4=50—59 N_5—60—69 For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parentheses and the S D for the differences

	CR_1 V	CR_2 V_2	CR_4 V_4	CR_5 V_5	CR V_7
N_1	0 0	0 0	10 06	18 16	18 18
	0	±0	(40 08	11) 08	0 0
N_2	0 0	0 0	06 06	18 20	18 18
	0	−0	(0 ±0	11 10	0 08
N_3	0 0	0 0	08 06	12 10	1? 14
	0	0	(2? 08	17) 04	17 04
N_4	0 0	0 0	04 02	06 06	04 04
	0	±0	(50 ±04	0 0	0 _0
N_5	0 0	0 0	08 10	16 14	14 12
	0	0	25 06	13) 06	14 06
N_{1-5}	0 0	0 0	10 08	14 14	14 14
	0	0	(20 04	0 06	0 04

TABLE 3 QRS interval (c sec) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_6 V_6	CR_7 V_7
N_1	8 8 8 6	9 6 9 2	8 8 8 8	9 0 9 2	8 0 8 2
	(2) ±0 4	(4) ±0 8	(0) ±1 0	(—2) ±1 4	(—3) ±1 6
N_2	8 6 8 0	7 6 7 8	7 8 7 6	7 0 7 2	7 6 7 4
	(7) ±1 4	(—3) ±1 0	(3) ±1 8	(—3) ±1 4	(3) ±1 2
N_3	9 0 9 0	9 2 9 6	8 0 8 2	8 2 7 8	6 8 6 0
	(0) ±1 0	(—4) ±0 8	(—3) ±0 6	(5) ±1 4	(12) ±1 0
N_4	10 0 10 2	10 0 9 6	9 8 9 4	8 4 8 0	8 0 6 8
	(—2) ±1 6	(4) ±1 2	(4) ±1 2	(5) ±1 4	(15) ±1 8
N_5	10 0 10 2	9 8 9 4	8 6 8 4	7 6 7 2	7 6 7 0
	(—2) ±1 0	(4) ±1 2	(2) ±0 8	(5) ±1 0	(8) ±1 8
N_{1-5}	9 2 9 0	9 2 9 2	8 6 8 4	8 0 7 8	7 6 7 2
	(2) ±1 2	(0) ±1 0	(2) ±1 2	(3) ±1 4	(5) ±1 6

TABLE 4 QT interval (c sec) in 5 age groups with 10 normal females and males in each decade (N_1=20—29, N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR V_1	CR V	CR_4 V_4	CR_6 V_6	CR V_7
N_1	37 0 36 4	36 8 36 4	37 0 36 8	36 6 37 0	36 2 36 4
	(2) ±3 2	(1) ±1 0	(1) ±1 2	(—1) ±0 6	(—1) ±1 2
N_2	37 8 37 2	37 0 37 2	38 0 37 0	33 4 33 2	37 0 37 0
	(2) ±2 0	(—1) ±0 6	(3) ±3 8	(1) ±1 2	(0) ±0
N_3	37 2 36 4	37 6 37 6	37 6 36 8	38 0 37 4	37 4 36 2
	(2) ±3 0	(0) ±0	(2) ±1 0	(2) ±2 6	(3) ±1 0
N_4	37 6 36 0	38 4 37 6	39 2 38 0	38 4 38 0	38 0 36 8
	(4) ±2 6	(2) ±1 8	(3) ±1 4	(1) ±0 8	(3) ±1 6
N_5	39 0 38 8	39 4 39 4	39 8 39 4	39 4 39 4	39 2 38 4
	(1) ±1 [illegible]	(0) ±1 0	(1) ±1 0	(0) ±0	(2) ±1 0
N_{1-5}	37 8 37 0	37 8 37 6	38 4 37 8	37 2 37 0	37 8 37 2
	(2) ±2 0	(1) ±1 0	(2) ±2 0	(1) ±0 8	(2) ±1 2

TABLE 5 P amplitude (mV) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49, N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR1 V1	CR2 V2	CR4 V4	CR5 V5	CR7 V7
N_1	21 10	15 10	16 07	12 05	14 07
	(52) ±19	(33) ±05	(56) ±03	(58) ±04	(50) ±04
N_2	12 10	14 11	14 08	10 05	12 07
	(17) ±04	(21) ±04	(43) ±05	(50) ±04	(42) ±04
N_3	14 11	13 09	17 09	14 08	15 08
	(21) ±05	(31) ±15	(47) ±03	(43) ±04	(47) ±01
N_4	14 10	17 11	16 09	14 07	12 07
	(29) ±04	(3?) ±05	(44) ±04	(50) ±04	(4?) ±03
N_5	13 09	15 09	16 10	12 06	13 05
	(31) ±05	(40) ±04	(38) ±07	(50) ±03	(62) ±03
N_{1-5}	15 10	15 10	16 09	12 06	13 06
	(33) ±1[illegible]	(33) ±04	(44) ±05	(50) ±04	(54) ±04

TABLE 6 Q amplitude (mV) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR1 V1	CR2 V2	CR4 V4	CR5 V5	CR7 V7
N_1	0 0	0 0	04 02	15 09	16 13
	±0	±0	(50) ±04	(27) ±04	(25) ±05
N_2	0 0	0 0	04 02	09 07	10 09
	±0	±0	(50) ±04	(22) ±06	(10) ±02
N_3	0 0	0 0	04 03	08 07	09 07
	±0	±0	(25) ±03	(13) ±04	(26) ±04
N_4	0 0	0 0	02 01	03 04	03 03
	±0	±0	(50) ±02	(—33) ±02	(0) ±0
N_5	0 0	0 0	03 04	09 08	09 06
	±0	±0	(—33) ±03	(11) ±02	(33) ±04
N_{1-5}	0 0	0 0	03 02	09 07	09 07
	±0	±0	(33) ±03	(22) ±04	(22) ±04

Table 7 R amplitude (mV) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_5 V_5	CR V_7
N_1	5 0 4 7	12 2 10 2	26 8 21 9	26 5 20 7	17 3 11 1
	(6) ±1 2	(16) ±1 9	(18) ±1 8	(22) ±5 1	(36) ±2 3
N_2	3 9 3 1	13 8 12 8	21 6 16 6	22 2 16 7	16 2 10 7
	(21) ±0 8	(7) ±1 7	(23) ±3 5	(25) ±3 7	(34) ±4 4
N_3	3 8 3 0	5 3 3 4	21 8 17 0	17 9 13 4	11 3 6 8
	(21) ±1 6	(36) ±2 0	(22) ±2 4	(25) ±2 2	(40) ±1 2
N_4	3 7 2 7	5 4 4 0	17 4 12 9	17 0 12 5	11 9 7 0
	(27) ±0 7	(26) ±0 7	(26) ±2 4	(26) ±1 8	(41) ±2 2
N_5	6 1 4 5	9 5 7 0	25 5 20 9	20 7 15 4	13 8 7 5
	(26) ±1 4	(26) ±1 5	(18) ±1 5	(26) ±1 3	(46) ±1 4
N_{1-5}	4 5 3 6	9 2 7 5	22 6 17 8	20 9 15 8	14 1 8 6
	(20) ±1 2	(19) ±1 6	(21) ±2 3	(24) ±2 3	(39) ±2 6

Table 8 S amplitude (mV) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V	CR_6 V_6	CR V
N_1	9 0 10 6	21 5 23 2	8 9 7 4	3 3 2 5	1 4 0 4
	(—18) ±4 0	(—8) ±3 2	(17) ±3 1	(24) ±0 9	(71) ±0 9
N_2	8 3 11 8	16 4 18 9	5 9 5 7	0 9 0 4	0 4 0 1
	(—42) ±3 5	(—15) ±2 5	(3) ±1 2	(56) ±0 7	(75) ±0 6
N_3	5 5 7 0	10 3 8 4	2 4 2 2	1 3 0 9	0 4 0 1
	(—27) ±4 1	(18) ±1 8	(8) ±0 9	(31) ±0 7	(75) ±0 6
N_4	9 3 11 1	12 3 10 6	8 0 7 7	3 0 2 1	0 7 0 2
	(—19) ±3 0	(14) ±3 3	(4) ±1 3	(30) ±0 9	(71) ±0 6
N_5	6 8 8 8	9 3 7 7	3 9 3 6	0 9 0 8	0 2 0
	(—29) ±1 9	(17) ±1 5	(8) ±1 2	(11) ±0 4	(75) ±0 5
N_{1-5}	7 9 10 0	14 0 13 8	5 8 5 3	1 9 1 4	0 6 0 2
	(—27) ±3 7	(1) ±3 1	(9) ±1 7	(26) ±0 8	(67) ±0 7

Table 9 ST J amplitude (mV) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59, N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_6 V_6	CR_7 V_7
N_1	08 08	24 21	08 08	02 01	02 0
	(0) ±05	(13) ±04	(0) ±06	(50) ±02	(100) ±04
N_2	10 08	21 21	06 05	04 03	03 01
	(20) ±06	(0) ±07	(17) ±04	(25) ±03	(67) ±04
N_3	09 11	12 15	02 03	01 01	0 0
	(—22) ±03	(—25) ±06	(—50) ±04	(0) ±0	±0
N_4	07 08	13 11	05 05	01 01	0 0
	(—14) ±05	(15) ±06	(0) ±02	(0) ±04	±0
N_5	10 10	13 13	07 04	01 01	0 0
	(0) ±07	(0) ±00	(43) ±04	(0) ±02	±0
N_{1-5}	09 09	17 16	06 05	02 01	01 0
	(0) ±05	(6) ±06	(17) ±04	(50) ±01	(100) ±03

Table 10 T amplitude (mV) in 5 age groups with 10 normal females and males in each decade (N_1=20—29 N_2=30—39 N_3=40—49 N_4=50—59 N_5=60—69) For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_6 V_6	CR_7 V_7
N_1	36 22	118 91	114 91	72 47	46 24
	(39) ±28	(23) ±01	(20) ±16	(35) ±13	(48) ±16
N_2	26 18	98 83	87 70	64 44	44 26
	(31) ±24	(15) ±16	(20) ±14	(31) ±11	(41) ±14
N_3	27 23	67 53	62 45	49 35	35 19
	(15) ±22	(21) ±11	(27) ±09	(29) ±16	(46) ±05
N_4	45 38	77 59	82 67	59 41	38 19
	(16) ±24	(23) ±11	(18) ±04	(31) ±08	(50) ±09
N_5	54 42	89 68	83 65	63 43	44 23
	(22) ±23	(24) ±04	(22) ±12	(32) ±10	(48) ±09
N_{1-5}	38 29	90 71	77 59	61 41	41 22
	(24) ±23	(21) ±13	(23) ±11	(33) ±10	(46) ±11

TABLE 11 Intervals (c sec) and amplitudes (mV) for 40 cases with ST and ST J changes For each electrode position the following values are given mean values for both CR and V leads the differences in per cent between CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_6 V_6	CR_7 V_7
Interval					
PR	16 6 17 0	16 6 16 8	17 0 17 2	16 6 17 2	16 6 16 8
	(—2) ±2 2	(1) ±1 4	(1) ±1 6	(4) ±1 4	(1) ±1 8
Q	1 8 1 8	1 6 1 6	0 8 0 6	1 2 1 2	1 2 1 2
	(0) ±0 4	(0) ±0	(25) ±0 8	(0) ±0 6	(0) ±0 8
QRS	8 6 9 0	8 6 8 6	9 2 9 0	8 6 8 4	8 4 7 8
	(5) ±1 2	(0) ±1 6	(—2) ±1 4	(2) ±1 2	(7) ±1 8
QT	35 4 35 0	36 2 35 8	36 4 36 2	36 4 36 0	36 8 37 6
	(1) ±3 2	(1) ±3 0	(1) ±1 8	(1) ±2 2	(1) ±1 8
Amplitude					
P	1 0 0 7	1 1 0 6	1 3 0 7	1 2 0 6	1 2 0 6
	(30) ±0 4	(45) ±0 6	(46) ±0 6	(50) ±0 5	(50) ±0 5
Q	0 5 0 6	0 9 0 9	0 5 0 4	0 8 0 7	0 6 0 4
	(—20) ±0 6	(0) ±0 1	(20) ±0 4	(13) ±0 4	(33) ±0 4
R	3 9 3 5	8 0 6 5	21 3 16 4	25 3 19 5	15 9 10 2
	(10) ±1 9	(19) ±2 2	(23) ±5 1	(23) ±4 1	(36) ±3 9
S	12 0 15 5	17 3 19 4	10 2 11 5	3 4 3 3	1 7 1 2
	(—29) ±4 7	(—12) ±4 8	(—13) ±4 5	(3) ±1 1	(29) ±0 9
ST J	0 0 4	0 0 2	—1 4 —1 0	—1 6 —1 2	—1 1 —0 4
	(—100) ±1 5	(—100) ±0 8	(—29) ±1 0	(—25) ±0 9	(—64) ±1 0
T	1 6 1 4	3 1 2 6	1 5 1 1	±0 ±0	0 3 —0 1
	(13) ±1 3	(16) ±2 0	(27) ±1 6	(0) ±1 3	(133) ±1 0

TABLE 12 Intervals (c sec) and amplitudes (mV) for 40 cases with myocardial infarction For each electrode position the following values are given mean values for both CR and V leads within parenthesis and the S D for the differences

	CR_1 V_1	CR_2 V_2	CR_4 V_4	CR_5 V_5	CR_7 V_7
Interval					
PR	16 8 16 9	16 6 16 4	16 4 16 2	16 6 16 4	16 4 16 6
	(1)±2 2	(1)±2 6	(1)±2 4	(1)±2 4	(1)±2 8
Q	0 6 0 4	0 6 0 6	1 6 1 4	1 8 1 6	1 6 1 6
	(33)±0 8	(0)±0 2	(12)±0 8	(11)±0 8	(0)±0 8
QRS	9 6 9 0	9 2 8 8	8 8 8 8	8 4 8 2	8 0 7 8
	(6)±1 4	(4)±1 4	(0)±1 2	(2)±1 4	(3)±1 4
QT	37 8 37 4	38 2 38 6	38 6 38 8	38 6 38 2	38 0 38 0
	(1)±3 0	(1)±2 2	(1)±2 2	(1)±2 0	(0)±2 2
Amplitude					
P	1 1 0 6	1 2 0 7	1 3 0 7	1 4 0 7	1 4 0 8
	(45)±1 2	(42)±0 5	(46)±0 6	(50)±0 5	(43)±0 5
Q	0 3 0 3	0 4 0 4	2 2 1 9	2 1 1 5	1 3 0 7
	(0)±0 2	(0)±0 1	(14)±1 3	(29)±1 1	(46)±0 9
R	1 6 1 7	3 9 3 0	8 2 6 3	14 2 10 1	11 6 7 8
	(—6)±1 0	(23)±2 8	(23)±4 3	(29)±3 0	(33)±2 6
S	11 0 13 5	18 4 20 9	9 2 8 4	3 5 3 1	1 0 0 6
	(—23)±3 1	(—16)±3 6	(9)±1 8	(11)±1 1	(40)±0 9
ST J	0 6 0 8	1 3 1 4	0 8 0 8	—0 3 —0 3	—0 1 0 1
	(—33)±1 1	(—8)±1 4	(±0)±1 9	(±0)±1 1	(—200)±0 8
T	3 1 3 0	4 9 4 6	—0 4 0 1	—1 8 —1 5	—0 3—0 2
	(3)±1 8	(6)±3 1	(—125)±2 7	(—17)±1 6	(—33)±1 4

References

1 Acheson R M Observer error and variation in the interpretation of electrocardiograms in an epidemiological study of coronary heart disease Brit J Prev & Soc Med 14 99 1960

2 Areskog N H and Hallen A ECG at rest and ECG response to exercise In A Hallén Angina pectoris Acta chir scand 70 Suppl 323 1965

3 Åstrand Irma Aerobic work capacity in men and women with special reference to age Acta physiol scand 49 Suppl 169 1960

4 Åstrand Irma Exercise electrocardiogram in a 5 year follow up study Acta med scand 173 257 1963

5 Åstrand Irma Exercise electrocardiograms recorded with an 8 year interval in a group of 204 women and men 48—63 years old Acta med scand 178 27 1965

6 Bengtsson E The exercise electrocardiogram in healthy children and in comparison with adults Acta med scand 154 225 1956

7 Blackburn H The electrocardiogram in cardiovascular epidemiology Problems in standardized application Ann New York Acad Sc 126 882 1965

8 Blackburn H Keys A Simonson E Rautaharju P Punsar S The electrocardiogram in population studies A classification system Circulation 21 1160 1960

9 Blackburn H Taylor H L Okamoto N Rautaharju P Mitchell P L and Kerkhoff A C Standardization of the exercise electrocardiogram A systematic comparison of chest lead configurations employed for monitoring during exercise, p 101 in Karvonen M J and Barry A J Physical activity and the heart Proceedings of a symposium Helsinki Finland C C Thomas Springfield Illinois 1967

10 Blomqvist G The Frank lead exercise electrocardiogram A quantitative study based on averaging technics and digital computer analysis Acta med scand 178 Suppl 440 1965

11 Blomqvist G and Åstrand Irma An American System for ECG Classification (in Swedish) Sv Läkartidn 60 2829 1963

12 Davies L G Observer variation in reports on electrocardiograms Brit Heart J 20 153 1958

13 Epstein F H Doyle J T Pollack A A Pollack H Robb G P and Simonson E Observer variation in interpretation of electrocardiograms Suggestions for objective interpretations J A M A 175 847, 1961

14 Evans W Which leads should we take? Amer Heart J 64 143 1962

15 Furberg C Adrenergic beta blockade and electrocardiographical ST T changes Acta med scand 181 21 1967

16 Grewin K E Some supplementary leads in clinical electrocardiography Acta med scand Suppl 209 1948

17 Grimby G and Saltin B Physiological analysis of physically welltrained middle aged and old athletes Acta med scand 179 513 1966

18 Holmgren A and Strandell T On the use of chest head leads for recording of electrocardiograms during exercise Acta med scand 169 57 1961

19 Kaijser L Ekg forandringar vid koronar insufficiens som funktion av arbetsintensitet och duration Sv Lakartidn 63 3340 1966

20 Karlefors T Exercise tests in male diabetics I Electrocardiographic study Acta med scand 180 Suppl 449 1966

21 Leatham A The chest lead electrocardiogram in health Brit Heart J 12 213 1950

22 Lundman T Smoking in relation to coronary heart disease and lung function in twins Acta med scand 180 Suppl 445 1966

23 Mattingly T The postexercise electrocardiogram Its value in the diagnosis of coronary arterial disease Amer J Cardiol 9 395, 1962
24 Olafsson O Rignér K G and Brante G Några kliniska resultat från Eskilstuna hälsokontroll 1964 To be published
25 Punsar S, Pyörälä K and Siltanen P Classification of Electrocardiographic ST segment Changes in Epidemiological Studies of Coronary Heart Disease Annual meeting of Finnish Medical Societies Abstract in Scand J Clin & Lab Invest 19 Suppl 95 112 1967
26 Rose G A The diagnosis of ischaemic heart pain and intermittent claudication in field surveys Bull WHO 27 645 1962
27 Rose G and Blackburn H Cardiovascular population studies Methods WHO Press Geneva 1967
28 Simonson E Differentiation between normal and abnormal in electrocardiography C V Mosby Co St Louis 1961
29 Strandell T Electrocardiographic findings at rest during and after exercise in healthy old men compared with young men Acta med scand 174 479 1963

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 482

HEMODYNAMICS IN EARLY ESSENTIAL HYPERTENSION

BY
PER LUND-JOHANSEN

BERGEN 1967

Hemodynamics in Early Essential Hypertension

FROM CHR MICHELSEN'S INSTITUTE AND MEDICAL
DEPARTMENT A, UNIVERSITY OF BERGEN
SCHOOL OF MEDICINE BERGEN NORWAY

Hemodynamics in Early Essential Hypertension

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BERGEN 1967

Preface

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Bergen June 1967

Per Le[illegible]

CONTENTS

Abbreviations

NT = normotensive(s)

HT = hypertensive(s)

BSA = body surface area (m^2)

VO_2 = oxygen uptake (ml/min/m^2 STPD)

STPD = standard temperature and pressure, dry

HR = heart rate (beats/min)

CO = cardiac output (l/min)

CI = cardiac index (l/min/m^2)

SV = stroke volume (ml/stroke)

SI = stroke index (ml/stroke/m^2)

BP = arterial blood pressure (mm Hg)

SAP = systolic arterial pressure (mm Hg)

DAP = diastolic arterial pressure (mm Hg)

MAP = mean arterial pressure (mm Hg)

TPR = total peripheral resistance (dyn sec.cm^{-5})

TPRI = total peripheral resistance index (dyn.sec cm^{-5} m^2)

LVWI = left ventricular work index (kpm/min/m^2)

LVSWI = left ventricular stroke work index (pm/stroke/m^2)

$(a-\bar{v})O_2$ = arteriovenous difference (ml/l)

CVP = central venous pressure (mm Hg)

kpm = kilopond meter

pm = pond meter

ECG = electrocardiogram/electrocardiography

* = $0.01 < P < 0.05$ = almost significant

** = $0.001 < P < 0.01$ = significant

*** = $P < 0.001$ = highly significant

I Introduction

In spite of the fact that hypertension – or more precisely – high arterial pressure in the systemic circulation – is very common in all parts of the world today, there are large gaps in our knowledge of the pathogenesis and etiology In a *minority* of hypertensive subjects, it is possible to demonstrate disease in the kidneys, endocrine system or the aorta which is responsible for the hypertension Even though we do not know exactly *how* the high blood pressure is established and maintained in these conditions, we at least know that in some patients the hypertension will be cured by surgery (i e extirpation of a diseased kidney or endocrine tumour) so that the pathological structure must in some way be responsible for the high blood pressure

However in the *majority* of hypertensive individuals, it is not possible with our present technique to detect any abnormalities which could be held responsible for the hypertension This condition is called 'essential hypertension Whether it is a qualitative disorder or simply quantitative is debated (125, 126, 127 128) The etiology of this common disorder is unknown and the pathogenesis is not clear

The arterial pressure in the systemic circulation is mainly determined by the volume of blood pumped into the vessels from the heart – *the cardiac output* – and the total diameter of the vessels (particularly of the arterioles) – *the total peripheral resistance*

What causes the high blood pressure in essential hypertension? It is well documented that, in *advanced* stages, the dominating disturbance is an increased peripheral vascular resistance due to reduction in the lumen of the arterioles The cardiac output is within normal limits or reduced This has been shown by measurement of cardiac output and total peripheral resistance by catheterization studies (22, 61, 65, 131 135, 155, 158, 164, 166) and less accurate methods (31, 47, 64, 67), by measurement of flow and resistance in parts of the systemic circulation (27, 28, 34 35) and by histological examination of the resistance vessels obtained by biopsy or in post mortem studies (103, 119 143)

These studies have led to the concept that the *primary* and cardinal disorder in essential hypertension is an increase in the total peripheral resistance mainly located in the arterioles (58) Many theories have tried to explain *why* the lumen of the arterioles becomes narrowed and extensive research has been performed to find substances or mechanisms which could reduce the arteriolar lumen Increased content of water and sodium in the vessel wall, increased reactivity to norepinephrine increased renin angiotensin–aldosterone activity and disturbances of the baroceptors

have been discussed, but none of these theories are generally accepted (92, 106)

However, not all subjects with essential hypertension have high peripheral resistance. Catheterization studies have shown that some subjects have a normal resistance and a high cardiac output (15, 16, 41, 42, 52, 53, 135, 158). Histological studies have shown that in the early phase of essential hypertension, the vascular changes so frequently found in later stages are scanty or lacking (103). Furthermore, observations from experimental renal hypertension in rats have shown that the cardiac output seems to be primarily involved in the establishment of the hypertensive state (54, 108, 109)

These observations have led to the theory that the increased vascular resistance in essential hypertension could be *secondary*, due to an initially increased cardiac output and autoregulation of the resistance vessels (40, 71, 108, 109)

If this concept is correct, it has great consequences for the search for the etiology of essential hypertension. The main question will then be turned from what could primarily cause reduction of the arteriolar lumen, to what could cause an increased cardiac output. The data in favour of this theory are few (93, 122), and in particular, *systematic* observations in humans are lacking

The need for such data has been stressed in expert reports from WHO (7)

This study is an attempt to fill the need for more data about the mechanisms responsible for the high systemic arterial pressure in essential hypertension in various stages, particularly in the earliest phase. Since essential hypertension is a disorder which runs a long course and most subjects are without symptoms in the earliest phase, a major problem is to get a representative group of patients. In the present study this difficulty was overcome thanks to a mass screening of the blood pressure in the adult population in Bergen in 1963 (44,153), and by collaboration with the health officers at some of the largest health centres in Bergen

Through this approach, a representative group of subjects with essential hypertension was found. Most of the subjects were without complications and, defined on the basis of this criterium, – in an early stage of the hypertensive process. However, since *the start* of the hypertensive process is the centre of our interest, it was necessary to find subjects in an early phase and with a short duration of the hypertension. Essential hypertension usually starts before the age of 50 years (13, 149). Since it was difficult to find subjects with a sufficient number of reliable blood pressure recordings to assess the duration of the hypertension with accuracy, subjects without complications in the age groups 17–30, 30–39, 40–49 years were studied (and also for comparative purposes, a group > 50 years where complications were frequent). It was assumed that the youngest group would mainly contain subjects with hypertension of short duration and the older groups, subjects with hypertension of longer duration. From the available data this assumption seems to be valid

The main purpose of this study is to determine whether the high systemic arterial pressure in early essential hypertension is caused by disturbances in the heart pump or in the peripheral vessels or by both factors in combination

The problem is attacked by *hemodynamic studies* of the systemic circulation: measurement of the cardiac output and intraarterial pressure, and calculation of the peripheral resistance. The measurements are performed on subjects with essential hypertension in early and later

phases and in controls with normal blood pressure

The results in the hypertensives are compared with the results in age matched normotensive controls to find out *if* and *how* the hemodynamic parameters deviate from normal The results in the youngest hypertensives are also compared with the results in the older hypertensives with longer duration of the hypertensive process, some also with complications

To make the groups as homogeneous as possible, the study was confined to men in whom essential hypertension is more serious than in women (104)

In the first part of the study, the hemodynamic situation is studied *at rest,* in the sitting and in the supine position

In the next sections the subjects are submitted to tests which involve a stimulus to the circulation and changes in the hemodynamic situation The tests are

1) *muscular exercise,* 2) *changes of the plasma volume* and 3) *food intake*

All these tests increase the cardiac output, and involve changes in the peripheral resistance The purpose of these tests is to see whether it is possible to detect disturbances in the cardiac or vascular response In the *first of these tests* the subjects are submitted to muscular exercise Strenuous muscular exercise is the greatest physiological stress to which the circulatory system can be exposed and it is probable that abnormalities in the circulatory system which are not present at rest can then be demonstrated Muscular exercise increases the cardiac output and the distribution of blood flow is greatly altered The blood flow in the working muscles increases up to 10-fold or more (75 76) and the blood flow to non working areas is reduced, for example in the renal (97, 129) and hepatosplanchnic (132) areas to 10% of the resting value During strenuous muscular exercise, most of the cardiac output passes through the active muscles where vasodilation takes place extensively If the ability for vasodilation is generally restricted in essential hypertension, then it should be revealed by strenuous muscular exercise

In the *second test,* one of the basic mechanisms in the regulation of the cardiac pump function is studied – namely Starling s law of the heart (150) In the intact organism the relationship between ventricular filling pressure and cardiac output is less than in the heart lung preparation, owing to interference by the autonomous nervous system If this is blocked by drugs, the cardiac response to changes in the filling pressure is greater (62) If it is assumed that disturbances in the autonomous nervous system could be responsible for the suspected high cardiac output in early hypertension, then this would be expected to result in a greater cardiac response to fluid loading A previous study supports this assumption but the data are few (157) However, much data has shown that the *renal* answer to such a test is greater in hypertensives than in controls (greater diuresis and natriuresis) (11, 18 37, 51, 79, 90 123, 156) It is possible that this simply reflects a different central hemodynamic response (157) It has been suggested that this augmented cardiac response could be secondary to a greater increase in the central venous pressure, pointing to a disturbance of the veins as well in early hypertension (157)

The *last test,* intake of a heavy meal, leads to a hemodynamic situation opposite to that in muscular exercise increased blood flow in the hepatosplanchnic area It might be expected that the systemic arterial pressure in this situation would be significantly influenced by the resist

ance in this vascular area. Since it has been stated that the vascular changes in essential hypertension appear earliest and are most pronounced in the reno-hepato-splanchnic region (65) the study of the hemodynamic changes in the postprandial phase seems important. Reports on such studies have not been available.

The *main part* of this work is devoted to studies *at rest* and *during muscular exercise*. The observations during changes in the plasma volume and after food intake must be interpreted as additional pilot studies.

The main problems which this study aims to elucidate can thus briefly be *summarized as follows*:

1 a) Is the high resting systemic arterial pressure in early essential hypertension caused by a high cardiac output or a high total peripheral resistance or by a combination of both factors?

b) Is the hemodynamic pattern in early essential hypertension different from that in later and advanced stages of the disorder?

c) Does the hemodynamic pattern in hypertensive subjects at various ages and stages point to a change in the hemodynamic disturbance in essential hypertension from a high flow normal resistance pattern in the early phase to a normal or subnormal flow high resistance pattern in the later stages?

2 a) Are the changes in cardiac output and total peripheral resistance induced by muscular exercise, changes in the plasma volume and by food intake different in subjects with essential hypertension compared to normotensives?

b) Are the responses in early hypertension different from those in the later stages?

c) Do the responses point to disturbance in the heart pump or in the total peripheral resistance or both?

d) Are the responses consistent with the assumption of a disturbed heart pump function in early essential hypertension together with normal peripheral vascular resistance?

II Subjects

A Selection of the subjects

1 The hypertensive group

All but 10 of the 93 hypertensives studied were selected from the Bergen survey (44, 153) and from the files at two of the largest health centres in Bergen[1]

In addition, *all* (10) men < 40 years with essential hypertension referred to the outpatient unit of Medical department A during the period of the study, were examined The subjects were studied hemodynamically if the following criteria were fulfilled

1 Sitting blood pressure > 140/90 at the mass screening at the last routine control by the health officer or other routine medical examination
2 Sitting blood pressure > 140/90 at follow up examination by health officer or physician
3 Sitting blood pressure > 140/90 at preliminary examination by the author
4 Untreated essential hypertension without heart failure or uremia
5 No other diseases (including obesity)
6 Willing to cooperate in the hemodynamic study

[1] The health centre for the police post customs and Fishing Laboratory departments and the health centre at Bergen Mekaniske Verksted (the largest shipbuilding yard in Bergen)

The cooperation of the hypertensives was most remarkable Only one of the 94 men who were asked if they would participate in the study hesitated initially All the others consented at once No attempt was made to persuade the subjects (It is interesting that the subject who declined called the investigator after two years, when he had heard about the investigation on television, and he then wanted to assist Since the statistical calculations were completed, the subject was not studied hemodynamically)

a) The diagnosis of essential hypertension

a1) *clinical examination* All subjects underwent a thorough clinical examination, usually by at least two physicians and including palpation of the foot arteries, auscultation for murmurs from the kidney arteries, and ophthalmoscopy

a2) *urine tests* specific gravity, albumin, blood glucose, sediments, bacterial count, and with a few exceptions, measurement of the excretion of catecholamines In a few patients the aldosterone secretion rate was also determined (111)

a3) *blood tests* hemoglobin (lowest recorded value was 12 [illegible] g/100 ml), creatinine (highest recorded value was 1,7 mg/100 ml), Na K and Cl (None had hypokalemia)

a4) *electrocardiography* a 12-lead ECG was taken in all patients and classified according to Sokolow and Lyon (148) However, in accordance with Sannerstedt (135), left ventricular hypertrophy was not diagnosed on the basis of elevated R-waves over the ventricle alone, but only if ST-depression and/or negative T-waves were present

a5) *x-rays* All subjects had a mass x ray of the chest. Roentgenographic study of the heart was performed in most patients but not in a few with only slight hypertension, normal ECG and negative physical examination Intravenous pyelography was performed in all but two patients (both had mild hypertension and negative urine at health control through many years) The test was carried out without compression during the first minutes of the excretory phase Renal arteriography was performed in about $^1/_3$ of the patients (if the pyelography was doubtful, or if the blood pressure was particularly high)

a6) *additional tests* Regitine test was carried out only if the hypertension was severe or if the history was suggestive of pheochromocytoma (performed in less than $^1/_3$ of the patients)

The diagnosis of essential hypertension was made if the history and these tests did not indicate a secondary hypertension (coarctatio aortae, pheochromocytoma aldosteronoma, or primary renal disorders)

b) Classification of the stage of hypertension

The stage of the hypertensive disorder was classified according to the criteria proposed by WHO (7) (table 1) It is seen that only 3 of the 72 patients < 50 years had any complication, and this was only minor cardiovascular hypertrophy The age group > 50 years was more heterogeneous and consisted mainly of patients with complications At the time of the study, all hypertensives were ambulant without clinical heart failure and all but 3 were in work

As stated in the introduction, it is rarely possible to assess the duration of hypertension Table 2 gives the known duration of hypertension in the subjects in stage I < 50 years The values are presented with great reservations, and it is probable that in many subjects, the hypertension had persisted considerably longer than is apparent from the table The table shows that at least 50% of the subjects > 40 years still in stage I had been hypertensive for at least a decade

Table 1 *Age distribution and hypertensive stage in the total group (Stage I = no complications Stage II = no complications except cardiovascular hypertrophy Stage III = evidence of organ damage)*

Age (years)	Normotensives	Hypertensives			
		Stage I	Stage II	Stage III	Total
17–29	18	21			21
30–39	19	19			19
40–39	11	29	3		32
50–66	0	6	10	5	21
Total	48				93

Table 2 *Known duration of hypertension in subjects in stage I aged 17–49 years (The figures in parentheses express the percentage of the total number in the age groups)*

Age (years)	n	≥ 5 years	≥ 10 years	≥ 15 years	No of subjects hypertensive before 30 years age
17–29	21	6 (29)	3 (14)	0	21 (100)
30–39	19	9 (47)	4 (21)	1 (5)	10 (53)
40–49	29	18 (62)	15 (52)	8 (28)	8 (28)

2 The normotensive controls

The normotensive control group consisted of 48 healthy individuals with a negative history, negative clinical examination and blood pressure below 140/90 In practically all of them, blood pressure readings taken more than two years earlier were available and they all had previous readings below 140/90 Most of the controls were recruited through a request for male volunteers at the registration centre for blood donors A large number responded including blood donors as well as many of their friends and aquaintances who had never given blood The hemodynamic study was not carried out until a minimum of 5 weeks had elapsed since the last donation Subjects who were actively training for sports competitions were not used

B Subjects studied at rest

All 93 hypertensives and 48 normotensives were studied at rest

1) Sitting group This group contains the majority of the subjects, 77 hypertensives (patients no 1–77) and 33 normotensives The group is the same as the exercise group + 9 hypertensives > 50 years who were studied at rest only The age distribution, body weight and BSA are shown in table 3 There were no significant differences in the body weight or BSA between hypertensives and their controls

2) Supine group This group contains 16 hypertensives (patients no 78–93) and 15 normotensives who were studied at rest, supine before dextran infusion or food intake As the number is small, the subjects < 40 years are included in one group

Table 3 *Age distribution body weight and BSA in the subjects studied at rest sitting and during exercise*

		Rest and exercise								Rest only	
		17–29 years		30–39 years		40–49 years		50–56 years		50–66 years	
		NT	HT	NT	HT	NT	HT	NT	HT	NT	HT
n		11	19	11	17	11	25	7		16	
Weight (kg)	Mean	72.2	71.3	75.5	79.5	75.5	73.8	77.7		77.3	
	SD	5.8	6.5	5.6	9.8	9.8	7.6	9.0		9.0	
BSA (m^2)	Mean	1.912	1.884	1.925	2.002	1.928	1.888	1.967		1.948	
	SD	0.119	0.097	0.075	0.142	0.136	0.103	0.130		0.130	

Table 4 *Age distribution, body weight and BSA in the subjects studied at rest supine*

	20–39 years		40–49 years		50–61 years	
	NT	HT	NT	HT	NT	HT
n	15	4		7		5
Weight (kg) Mean	72.1	80.0		75.6		76.4
BSA (m²) Mean	1.891	1.993		1.864		1.890

The age distribution, weight and BSA are shown in table 4. (The statistical calculations are omitted owing to the small number.)

C. Subjects studied during exercise

This group contains 68 hypertensives and 33 normotensives (the rest sitting group less 9 hypertensives, patients no. 63, 64, 67, 72, 77). The age distribution, weight and BSA are shown in table 3. All but one of the subjects < 50 years were in stage I. Of the 7 subjects 50–56 years old, 4 were in stage I, 2 in stage II and 1 in stage III.

Since the cardiovascular response to exercise is dependent upon the training of the subject (60), an attempt was made to classify the habitual physical activity of the subjects as high, medium or low according to the following criteria:

High: heavy work or regular physical exercise twice a week or more.

Medium: work involving some physical activity or more than 1 hr walking outdoor daily and usually more than 3 hrs walk on Sundays.

Low: sedentary work, less than ½ hr walking outdoor daily, rarely or never longer trips on Sundays.

The results are shown in table 5. There is no great difference to be seen between the hypertensives and the controls in the two youngest age groups. In the two oldest more hypertensives than controls had "low" activity, yet the majority in the age group 40–49 years had "high" or "medium" activity level. It was rather surprising that so few had "low" activity. Surprisingly many walked to or from their place of work and surprisingly many took regular Sunday trips. The facilities for outdoor walking are excellent in Bergen with mountains close to the city. This might be reflected in the results. In a cross country walking competition in 1966 involving a distance of 25 km and about 800 m climbing, 10 500 of the population (about 110 000) participated!

All subjects in the exercise group had used a bicycle previously.

Table 5 *Physical activity in the subjects studied during exercise. (The figures in parentheses are per cent values.)*

	17–29 years		30–39 years		40–49 years		50–56 years	
	NT	HT	NT	HT	NT	HT	NT	HT
Total group n	11	19	11	17	11	25		7
High activity n	5(45)	8(42)	4(36)	5(29)	3(27)	5(20)		0 (0)
Medium activity n	3(27)	7(37)	5(46)	7(41)	7(64)	13(52)		3(43)
Low activity n	3(27)	4(21)	2(18)	5(29)	1 (9)	7(28)		4(57)

Table 6 *Body weight and BSA in the subjects studied during dextran infusion and food intake*

		Dextran studies				Meal studies	
		< 40 years		> 40 years			
		NT	HT	NT	HT	NT	HT
	n	10	9		[illegible]	5	7
Weight (kg)	Mean	71 0	76 0		78 1	74 1	71 0
	SD	5 6	9 6		9 1	—	—
BSA (m²)	Mean	1 884	1 952		1 892	1 900	1 850
	SD	0 095	0 153		0 124	—	—

D Subjects studied after dextran infusion

The hypertensive group consisted of 18 men aged 19–59 years divided into two groups 9 below 40 years, all stage I (patients no 4 5 13 16 78 79 18, 25 and 80) and 9 above 40 years 5 in stage I and 4 in stage II (patients no 38 81, 44 82, 83, 55, 84, 85 86) Patients no 78–86 had not been investigated before the remainder had taken part in work experiments 2–3 months previously The normotensive controls were 10 men aged 20 38 years The body weight and BSA are shown in table 6 and are not significantly different in the three groups

E Subjects studied after food intake

Seven hypertensive men aged 35–61 years (patients no 87–93) — 4 in stage I and 3 in stage II, and 5 controls aged 25–35 took part in this experiment None had been examined hemodynamically before Body weight and BSA are shown in table 6 (Owing to the small number, statistical calculations are omitted)

III Methods

A Technical

1 Oxygen uptake Oxygen consumption was measured by the conventional Douglas bag and a low resistance open circuit system Samples of expired air were analysed by the Scholander ½ cc method (138) The volume of expiratory air was measured by a gasometer calibrated frequently by a bell spirometer, and the volume converted to STPD

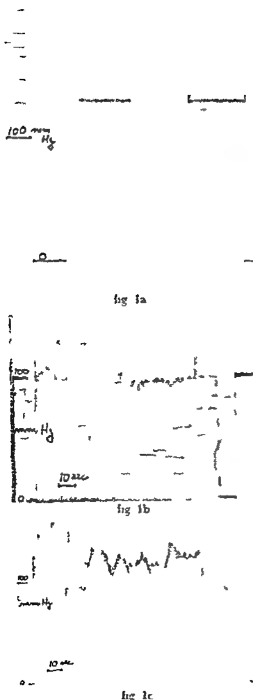
2 Heart rate The heart rate was recorded by electrocardiography (Elema Schonander Minograf 41) The ECG arterial pressures and the dye dilution curve (see later) were recorded on a multichannel oscillographic recorder (ABEM Ultralette)

3 Brachial artery blood pressure The systemic arterial blood pressure was recorded intraarterially by a thin (external diameter 1 56 mm, internal diameter 1 15 mm) polyethylene catheter introduced into the brachial artery and connected to an Elema Schonander strain gauge manometer (Elektromanometer 190 A) The instrument was calibrated and checked for linearity with water in a glass tube Zero level in the supine position was the mid axillary line and in the sitting position, a horizontal plane through the sternal insertion of the 4th rib The systolic, diastolic and electrically integrated mean pressures were recorded during several respiratory cycles and free hand smoothing of the curves was used to obtain the pressure values presented (91)

In some subjects, particularly the young hypertensives, the blood pressure could vary considerably over a few seconds, thus making registration of the pressures during

Table 7 *Difference between brachial artery pressure recorded intraarterially and externally in 35 hypertensives mm Hg (figures in parentheses are per cent values)*

Age years	n	SAP			DAP		
		Intraart	External–Intraart		Intraart	External–Intraart	
		Mean	Range	Mean	Mean	Range	Mean
<40	13	148 7	−12 — +4	− 3 5(−2 3)	85 5	−5 — +13	+4 3(+5 0)
40–49	17	161 3	−15 — +22	2 2(−1 4)	93 1	−4 — +13	+5 9(+6 3)
50–56	5	183 2	−21 — −9	−15 2(−8 3)	100 4	0 — +18	+4 9(+4 9)
Total	35	159 7	−21 — +22	− 4 5(2 8)	91 3	−5 — +18	+5 1(+5 6)



20–30 seconds necessary Examples of a stable and a very labile pressure curve together with a recording of the pressure from a mechanical pump are shown in fig 1 As only one arterial catheter was used the pressure and cardiac output could not be recorded simultaneously but the pressures were taken immediately before registration of the dye curves

a) Comparison of intraarterial and external blood pressure measurement

As routine measurement of blood pressure was performed by the conventional cuff method it was of interest to compare the pressures obtained by the two methods The procedure was carried out as described on page 43 The mean value of three external measurements was compared with the intraarterial pressures obtained by free hand smoothing of the intraarterial curve obtained between the first and the last measurement The results from 35 hypertensives in whom the comparison was made are shown in table 7 Usually agreement between the two methods was good as reported by others (89) The mean value of the external systolic pressure was 4 5 mm Hg (2 8%) too *low* and the diastolic pressure 5 1 mm Hg (5 6%) too *high* by comparison with the intraarterial values (External diastolic pressure was recorded at the point when the sound disappeared)

4 **Central venous pressure** (measured only in the plasma expansion and the heavy meal experiments) was measured by saline in a vertical glass tube connected to a catheter advanced from a cubital vein to the region of the superior vena cava The zero level was the mid axillary line (only supine measurements)

Fig 1 Pressure recording from a circulatory model (a) from the brachial artery in a normotensive subject (b) and the brachial artery in a hypertensive subject with a very labile blood pressure (c)

Table 8 *The error of single CO determination calculated from 15 successive duplicate determinations at rest and during work load (in controls)*

	Rest	Work Load (kpm/min)		
		300	600	900
$CO_{mean} = \frac{\Sigma (CO_1+CO_2)}{2n}$ (l/min)	6 68	13 19	17 39	23 68
Error (l/min)	± 0 28	± 0 48	± 0 84	± 1 07
Error in % of the mean	± 4,2	± 3 6	± 1 8	± 4 5

5 Cardiac output The cardiac output was recorded by the dye dilution method using a linearly responding monochromatic densitometer and recording continuously in arterial blood The withdrawn blood was reinfused (An initial attempt to use the ear piece method (63), was unsatisfactory) The instrument, constructed at Chr Michelsen s Institute Bergen, has been described in a previous paper (101) Exactly 2 ml of a 2 5 mg/ml solution of Cardiogreen was injected through a 60 cm long catheter of the same diameter as the arterial catheter advanced to the region of the axillary vein or superior vena cava The dye in the catheter was flushed into the vessel with 10 ml saline (23) Calibration of the curves was carried out before all registrations in new situations with the exception of the 900 kpm/min load As the calibration procedure took 1–2 minutes, it was usually omitted at this work load and the calibration factor from the 600 kpm/min load was used There was no consistent difference between the calibration factors at 600 and 900 kpm/min The curves were plotted on semilogarithmic paper, and the cardiac output calculated according to Hamilton s principle (77 117)

All plotting and calculating was done by the author

With a few exceptions, double analyses with approximately 2 min interval were performed, and the mean value used in the calculations The reproducibility of the values obtained in steady state conditions with 2 min interval was good The error of single determination, including the biological variation, was less than ± 5% (during exercise as well) calculated from the formula $\sqrt{\frac{\Sigma (d)}{2n}}$, where d is the difference between duplicate determinations and n the number of duplicate determinations The results of the double analyses are shown in table 8

a) Model experiments for controlling the method for determination of cardiac output

Since the most critical measurement in this study is determination of the cardiac output, and since the instrument in use has so far not been used outside this laboratory, it was considered important to test the reliability of the instrument and the calibration method employed In collaboration with E Hatleveit at Chr Michelsen s Institute, a simple circulatory model was constructed The "heart" was a plastic pump driven by an electromagnetic motor The frequency and stroke volume could be regulated within ranges similar to the human heart up to a flow of 12 l/min The pump was connected to rubber tubes of 16 mm internal diameter and a reservoir altogether a volume of approximately 5 6 l (a normal blood volume) and the system was filled with

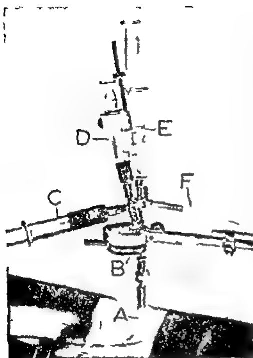


Fig 2 The injection system (A—venous catheter B=3 way valve C=syringe with supply of dye D syringe with stopper with dosage of dye E—syringe with saline heparin solution for flushing F—tube to supply of saline heparin in a bottle 3 way stopcocks on C/D and E/F

heparinized filtered ox blood In order to obtain accurate measurements of the flow a special valve was constructed and connected to an electrical stop watch When the flow was switched from the circulating system to the measurement cylinder the precise time was measured and the precise flow could be calculated from the volume in the cylinder and the time

Simultaneous control measurements of flow during dye injection would eliminate the recirculation and in addition require larger quantities of blood than are usually obtained from one ox Mixing of blood from several animals was to be avoided because of possible formation of aggregations In order to control the stability of the flow during dye injections a propel flowmeter[1] with a counter[2] was introduced into the system This showed flow variations with an accuracy within $\pm$ 10 ml/min in the actual flow range Before dye curves were obtained, the blood was allowed to circulate through the system for approximately 5 minutes in order to remove air bubbles A polyethylene catheter of the same type as the catheter used in human beings was introduced into the afferent tube ('large vein) and connected with the injection device used in human experiments This is shown in fig 2 Syringe E (for the afterflush) was filled with blood instead of saline Another catheter was introduced into an efferent tube (artery) This catheter was connected to a three way stopcock one lumen leading to the strain gauge transducer, the other to the densitometer The speed of the withdrawal pump was 15 ml/min

The Cardiogreen solution was prepared immediately before the measurements One batch of solution was made and calibration carried out by micropipetting 20 μl of this solution to exactly 10 ml of blood from a specially constructed syringe This gave a concentration of 5 mg/l The flow was set at different rates between 3 and 12 l/min and 3–4 curves were registered at each flow level The blood was changed frequently to make sure of being within the linearity range of the densitometer (0–10 mg/l) (101)

In order to check the equipment for lower flow rates measurements were also performed with a glass model of aorta with renal arteries connected to the model These experiments are described in a previous paper (121) but one representative series of results is presented here (table 10)

Results A typical dye curve from the model experiments is shown in fig 3 Both at the high and low flow rates the differences between the measurements of flow by the dye dilution method and the true flow were very small The greatest difference was less than $\pm$ 3 per

[1] Meter Flow , Ltd M 4/1000/60 Feltham Middlesex England

[2] Counter Frequency Meter TF 14–17 Marconi Instr Ltd England



Fig 3 Dye dilution curve from model experiment

cent of true flow as seen from tables 9 and 10

Thus the model experiment shows that the densitometer with the calibration method used gives an accurate determination of pulsating flow in the range from 0 5 12 l/min in in vitro experiments (Later comparison with Fick's method (*in humans*) showed good agreement (102

6 **The ergometer bicycle** used in the exercise studies had an armchair instead of a saddle, and mechanical brakes which were calibrated regularly (3) Pedalling rate 60 pr min As this type of cycle has no mechanisms to compensate for irregularities in the pedalling rate, minor deviations from the desired load were inevitable The armchair was chosen for safety reasons as fainting episodes could be expected (17, 45 135) One drawback is that the results cannot be directly compared with findings obtained on a saddle seat (see appendix)

B Experimental procedure

1 General

All subjects were made familiar with the laboratory on a previous visit and the purpose of the experiment was explained

Table 9 *Accuracy of dye dilution method in model experiments Single determinations High flowrates (3 8 11 7 l/min) (l/min)*

Flow by flowmeter	3 85	3 87	3 86		6 36	6 38	6 25	
Flow by dilution method	3 82	3 90	3 82		6 34	6 34	6 42	
Difference	−0 03	+0 03	−0 04		−0 02	−0 04	+0 17	
Greatest difference in %							+2 7	
Flow by flowmeter	8 51	8 49	8 52	8 60	11 5	11 4	11 7	11 7
Flow by dilution method	8 34	8 41	8 50	8 62	11 2	11 1	11 9	11 9
Difference	−0 17	−0 08	−0 02	+0 02	−0 3	−0 3	+0 2	+0 2
Greatest difference in %						−2 6		

Table 10 *Accuracy of dye dilution method in model experiments Single determinations Low flowrates (Renal artery) (1000 500 ml/min) (ml/min)*

Flow by flowmeter	510	495	880	930	940	1085
Flow by dye dilution	514	485	869	935	949	1075
Difference	+4	−10	−11	+5	+9	−10
Greatest difference in %		−2			+1 0	

They were given written instructions to take a light breakfast at 7 30 a m (2 slices of bread without butter with jam One glass of juice No tea, coffee or milk) Smoking was not allowed after 8 00 a m They met in the laboratory at 9 00 a m and emptied the bladder

Using the Seldinger technique (139) and local anesthesia a thin polyethylene catheter (external diameter 1,56 mm) was introduced without fluoroscopy via a medial vein to the region of the superior vena cava Another catheter was introduced by the same technique 10–15 cm into the brachial artery A low curtain screened the arm from the patient s view during the catheterization but the operator could observe the patient s face To relieve possible nervous tension in the patient conversation about indifferent topics was kept up during the catheterization The venous catheter was connected to the injection device The arterial catheter was connected to a threeway stopcock Both catheters were flushed with heparin in saline (the venous catheter 0 5 ml heparin (2500 international units) in 500 ml and the arterial catheter 2 5 ml heparin in 500 ml saline) approximately every 15 min When the catheters were introduced and fastened with tape and all traces of blood removed the subjects were shown what had been done so far It was demonstrated that subsequent procedures would only be manipulation with the stopcocks and that this would be without any sensations or discomfort to the subject The curtain was then placed in the screening position with the comment that the test subject would relax better if he was unaware of the registrations

The syringes with the dye were discretely hidden since the knowledge of a dye going to be injected could have some psychogenic effect which might influence the circulation

These details are mentioned because they are of major importance if the resting values shall not be meaningless It is particularly important when studying subjects like hypertensives with a labile circulation, which is very easily influenced by psychogenic stimuli as evident from page 43

The subject then rested comfortably, supine, for 30 minutes covered by a blanket if required The temperature in the room was 20–22 C Great care was taken to keep a quiet atmosphere in the laboratory Nobody except the assisting nurse and the technical assistant was allowed to enter the room

After the rest period of 30 minutes the procedure was different in the different experiments Those who took part in dextran infusion or heavy meal experiments continued to rest supine for another 30 minutes

The majority 33 controls and 77 hypertensives were placed in the chair of the ergometer bicycle (fig 4) and sat there for approximately 30 minutes before the measurements started During this period a sham resting measurement was performed including collection of air in the Douglas bag

In *all* experiments care was taken to ensure that the subjects did not sleep when measurements were taken The general condition of the subjects during the experiments was very good No fainting episodes occurred Only two subjects had transient pallor and nausea during the catheterization but both were completely well within 5 minutes

When the catheters were taken out manual compression was performed for 15 minutes by the author firm enough to make the radial pulse weaker Thereafter an elastic bandage was firmly applied for 30 minutes The compression was then loosened a little so that the radial pulse

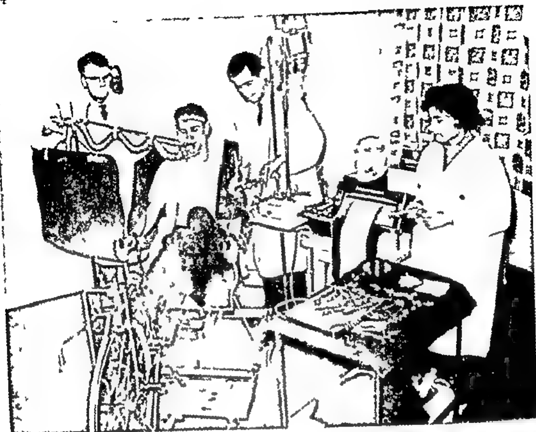


Fig. 4. The experimental set up

was equal to that on the other arm and the subject left the hospital. He removed the bandage himself in the evening. The subjects were instructed to call the investigator at the hospital or at home if they experienced pain, swelling or any abnormal sensations in the arm. Three called complaining of cubital tenderness or tingling in the fingers. One of them had a subcutaneous hematoma, the other two only presented tenderness at the puncture site. They were all treated with Tanderil[1] for 4 days and recovered without any sequelae or pulse differences between the two arms.

[1] Tanderil Geigy (Oxiphenbutazon)

2. Studies at rest

VO_2 (sitting group only), HR, CO, SAP, DAP and MAP were measured 60 min after catheterization. Collection time for expiratory air was 4 min.

3. Studies during exercise

After VO_2, HR, CO, SAP, DAP and MAP had been recorded sitting at rest, the subjects performed bicycling for periods of 8–9 min at 300, then 600 and finally 900 kpm/min with approximately 15–20 min intervals. Seven did not perform the 900 kpm/min work load: one normotensive subject in the oldest age group and one hypertensive in each of the three youngest age groups (all for technical reasons), and

300 mmHg

200

100

0

1'

2'

3'

6'

Fig 5 Intraarterial blood pressure before and during 900 kpm/min load in a hypertensive subject The recorder speed is changed during work Arrow marks start of cycling The small deflections represent electrically damped curves (for MAP)

1sec

1sec

1sec

1sec

Fig 6 Typical dye dilution curves from a) rest b) 300 kpm min c) 600 kpm min and d) 900 kpm min loads in order from left to right

the three oldest patients (fear that the load would be too heavy) Collection of expired air started after 5 min of cycling, and lasted for 1–3 min During the collection period, the heart rate and blood pressure were recorded continuously apart from two interruptions of the latter for measurement of the cardiac output The subjects continued to breathe through the mouth piece until the hemodynamic parameters were recorded Typical recordings of BP and CO during a work experiment are shown in fig 5 and 6

4 Studies after dextran infusion

CVP, HR, CO, SAP, DAP and MAP were measured 60 min after catheterization 500 ml 6% dextran in saline ("Macrodex" Pharmacia) were then infused through the venous catheter for 20 min New measurements were carried out (lasting about 10 min) Thereafter another 500 ml was infused (20 min) and new recordings taken as before 500 ml blood were then tapped through the arterial catheter over a period of 10–15 min, and 10 minutes later, new measurements were taken

5 Studies after food intake

CVP, CO, HR, SAP, DAP and MAP were measured 90 min after catheterization The catheters were then filled with heparinized saline and fixed to the arm with tape The individual sat on the examination bench supported by pillows and had his meal assisted by a nurse The meal consisted of

1) 200 ml cauliflower soup with one boiled egg
2) 2 large pork cutlets, fat sauce 3 boiled potatoes and vegetables (carrots and cauliflower)
3) 200 g ice cream (vanilla) 1 glass of orange juice

No smoking was allowed

All ate with a good appetite but a f[…] did not manage to eat all the ice crea[…] All were more than satisfied when finish[…] The meal took about 40 minutes Aft[…]wards, the individuals rested supine […] 20 minutes and the hemodynamic m[…]surements were performed as before

C Calculation and statistics

The parameters were converted to in[…] values corrected for body surface area […] follows

VO_2 ml/min/m
CI (= CO/BSA) l/min/m²
SI (= CI/HR) ml/stroke/m²
TPRI (= MAP × 80/CI) dyn sec cm[…] m² (80 = approximate conversion f[…]tor 1 332 × 60 = 79 92 ≈ 80)

$$LVWI \left(= \frac{CI \times MAP \times 13\,6}{1000}\right) kpm/min/\ldots$$

$$LVSWI \left(= \frac{SI \times MAP \times 13\,6}{1000}\right) pm/stro\ldots$$

The decision to express the data as ind[…] values needs some comments In clini[…] research, it is common to use the body su[…]face area as an indication of body size a[…] to express the CO in relation to BSA, t[…] so called cardiac index (76, 84, 159) U[…]fortunately however, the correlation b[…]tween the CO and BSA is not usually ve[…] strong, and the use of the BSA as the co[…]rection factor has been criticized seve[…] times (76) recently by Smulyan *et* […] (146) In the present work, the correlati[…] coefficient between CO and BSA in t[…] normotensives was 0 51 and as seen fro[…] fig 7 the CO in large subjects was usua[…] greater than in the smaller ones The co[…]relation between body weight and C[…] was slightly less (0 47) More complicat[…]

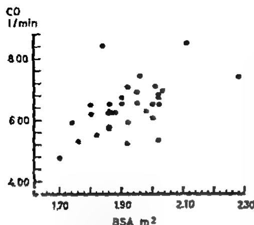


Fig 7 The relationship between CO and BSA in 33 normotensives aged 17–49 years (sitting) (r = 0 5)

correction factors have been proposed (146) but they have so far not been used to any extent In order to be able to compare the present results with the majority of those in the hemodynamic clinical literature (76 84, 159) the author chose to use the BSA as correction factor for the blood flow During muscular exercise, however, the CO is more dependent upon the work load than on the BSA (14) and it might then appear illogical to express the blood flow as CI It has been done, however, in order to be consistent and not to use CI at rest and CO during work For the same reasons, oxygen consumption is expressed per m² BSA

It is well known that there is little difference in the systemic arterial pressure in large and small mammalians and large and small humans The correlation between the MAP and the BSA in the normotensives in this study was only 0 2

In this work the main interest is focused around the peripheral resistance or the total arteriolar diameter As an indication of this the so called total peripheral resistance is used This is an expression of Ohm s resistance to laminar, non pulsative flow in straight rigid tubes (124) and is calculated as the ratio between MAP and CO The application of this expression to pulsative flow in human arteries is a very course approximation and must be interpreted with caution Within its limitations the parameter is of interest, since it characterizes the ratio between pressure and flow in a subject However since the flow is dependent upon the size of the subject and the pressure is nearly independent of the size, this ratio MAP/CO will indicate that if a small and a large man both have the same MAP, and the CO is higher in the large man than in the small (which is likely) then the total peripheral resistance will be greater in the small man than in the large man But the important question is whether or not the total peripheral resistance is too high *in relation to the body size* If instead we use the ratio MAP/CI this will indicate whether or not the diameter of the resistance vessels (53) is normal in relation to the body size Therefore the total peripheral resistance in this work is expressed as the ratio between MAP and CI and termed total peripheral resistance index (TPRI)

Statistics

The data were transferred to punch cards and fed into an IBM digital computor for statistical analyses The difference between the results in the hypertensives and in the corresponding controls was tested by Students T test In addition the effect of aging upon the hemodynamics sitting at rest and during exercise was tested by single variance analysis separately for normotensives and hypertensives (Owing to the small numbers the exercise results in the 7 hypertensives > 50 years were excluded from the variance analysis, and instead compared with the results in the hypertensives aged 40–49 years by the T test)

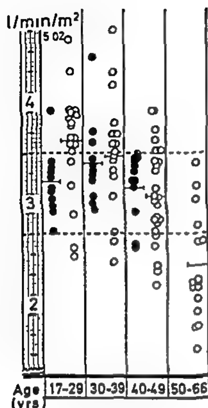


Fig 9 Cardiac index at rest sitting (Legend as in fig 8)

is *not* corrected for BSA, the CO in the youngest hypertensives is 6 96 ± 0 98 l/min, as against 6 31 ± 0 91 in the controls This difference is not statistically significant (P = 0 09) but the tendency is the same as for the CI In the age groups 30–39, 40–49 and 50–66 years, the significance of the difference from the controls is the same as for the CI values The significance of the differences between the hypertensive age groups also remains the same The possible *causes* of high cardiac output in the young hypertensives will be considered in the general discussion

In this chapter only the question of whether it is due to disturbances in frequency or stroke volume will be analysed

Table 13 *Heart rate at rest sitting (beats/min) (Age groups number of subjects and legend as in table 12)*

Age group	Mean SD NT	HT
1	68 1 7 9	79 1** 11 8
2	67 8 6 3	80 8** 13 4
3	67 7 8 8	76 7* 12 8
4		77 1 13 2

c) Heart rate (Table 13 and fig 10)
Normotensives The mean value in the total normotensive group is 67 9 beats per minute, the same as in the control group in Sannerstedt s study (135) These values are rather low compared with other catheterization studies (53, 59, 118, 154) and

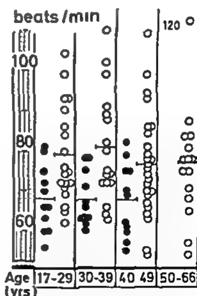


Fig 10 Heart rate at rest sitting (Legend as in fig 8)

probably reflect the quiet atmosphere in the laboratory and relatively good physical condition of the subjects There is a slight trend towards a decrease in the HR with aging but it is not significant

Hypertensives The hypertensives tend to have *high* heart rates and many have heart rates exceeding 82, the highest value in the control groups The difference from the control values is significant in the two youngest groups almost significant in age group 40–49 years, but not significant in the oldest group As in controls, there is a slight drop in HR with aging but it is not significant Similar findings have also been reported by Sannerstedt (135) Fejfar *et al* (52) and Konig *et al* (105) whilst other earlier investigations have shown varying results Finkielman *et al* (53) and Bello *et al* (15 16) report normal HR in hypertensives

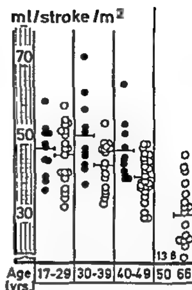


Fig 11 Stroke index at rest sitting (Legend as in fig 8)

d) Stroke volume (Table 14 and fig 11)
Normotensives The mean SI in the normotensive group is 49 9 ml/stroke/m², close to the value obtained by Sannerstedt (135) (46 5 ml/stroke/m² if corrected for BSA) There is no significant decrease in the SI with aging

Table 14 *Stroke index at rest sitting (ml/stroke/m²) (Age groups number of subjects and legend as in table 12)*

Age group	Mean SD NT	Mean SD HT
1	48 8	47 3
	6 4	7 4
2	52 3	44 7*
	9 1	5 8
3	48 5	42 0**
	6 9	5.5
4		32 9***
		8 5
		1–4***
		2–4**
		3–4**

Hypertensives The hypertensives tend to have *lower* values than the controls The difference from the controls becomes more and more marked with increasing age from not significant in the youngest to highly significant in the oldest age group The mean values decrease significantly with increasing age

Sannerstedt (135) also found no significant difference between the SV in hypertensives in stage I and in controls A decrease in the SV in advanced stages in hypertensives has also been reported by others (135, 158) In the early phase of essential hypertension, the reports on the SV have been varied Some workers (41 42, 53) have found that the SV was high in early essential hypertension The last observations however, were made in the supine position

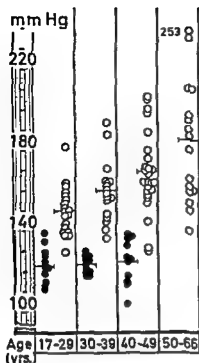


Fig 12 Systolic arterial pressure at rest sitting (Legend as in fig 8)

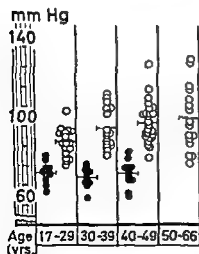


Fig 13 Diastolic arterial pressure at rest, sitting (Legend as in fig 8)

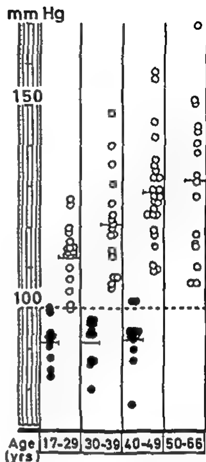


Fig 14 Mean arterial pressure at rest sitting (Legend as in fig 8)

e) **Brachial artery pressure** (Table 15 and figs 12–14)

Normotensives The mean values for the systolic, diastolic and mean pressure in the total normotensive group are 123,8–75 7 and 91 9 mm Hg respectively, very close to the values in normotensive Swedish controls (135)

In other studies, the mean values for the brachial artery pressure in normotensive controls have varied between 124/76 and 137/81 (59, 72 151)

Table 15 *Brachial artery pressure at rest, sitting (mm Hg) (Age groups number of subjects and legend as in table 12)*

Age group	SAP Mean SD NT	HT	DAP Mean SD NT	HT	MAP Mean SD NT	HT
1	122 8	150 2***	76 5	92 4***	91 9	112 7***
	8 2	11 8	4 9	6 8	5 4	7 5
2	123 5	159 9***	74 5	99 0***	91 5	120 9***
	4 2	14 6	4 8	9 7	5 3	11 9
3	125 0	168 8***	76 1	101 6***	92 5	128 3***
	10 7	19 5	6 4	10 2	6 7	12 5
4		184 3***		103 9***		131 1***
		30 9		15 6		17 6
		1–3*		1–3*		1–3**
		1–4***		1–4**		1–4***
		2–4***				2–4*

There is a tendency for the pressure to rise with increasing age but, as stated the differences between the groups are not significant

Hypertensives Figs 12 and 13 show that there is some overlapping in the SAP and DAP values in hypertensives and controls but fig 14 shows that the hypertensives and normotensives are quite well separated at a mean arterial pressure of 100 mm Hg The pressures in the hypertensives increase significantly with age but it can be seen that most of the hypertensives in the two oldest groups have mean pressures within the same range as those in the two youngest

f) Total peripheral resistance index (Table16 and fig 15)

Normotensives The mean value in the total normotensive groups is 2219 dyn - sec cm $^{-5}$m^2

The value is slightly lower than reported by Sannerstedt (135) (2427 dyn sec cm^{-5} m^2) There is a tendency towards an increase in the TPRI with aging after 30 years but it is not significant

Hypertensives Fig 16 shows that all but two hypertensives over 40 years have

Table 16 *Total peripheral resistance index at rest sitting (dyn sec cm^{-5}m^2) (Age groups number of subjects and legend as in table 12)*

Age group	Mean SD NT	HT
1	2247	2486
	183	404
2	2111	2761***
	240	481
3	2299	3283***
	266	521
4		4414***
		888
		1–3**
		1–4***
		2–3*
		2–4***
		3–4***

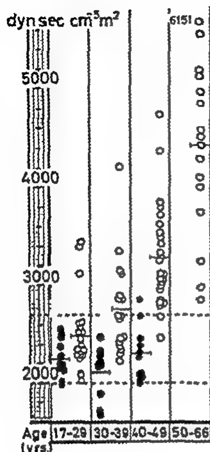


Fig 15 Total peripheral resistance index at rest sitting (Legend as in fig 8)

a high resistance index (above 2700 dyn - sec cm^{-5}m^2), as expected However, the majority of the hypertensives under 40 years have *lower* values

In the *youngest* group *only 3 of 19 have clearly elevated values*, and in this group, the *difference from the controls is not significant*

In the three other groups, the hypertensives have a highly significantly higher resistance index than their controls, but in the age group 30–39 years there are still many subjects with values within normal limits The TPRI increases significantly with aging The significance of the difference between the age groups is greater than for any of the other parameters

Nor did Fejfar *et al* (52) find any significant difference between the total peripheral resistance in young students with essential hypertension and normotensive controls Sannerstedt (135) found no significant difference between the TPR in hypertensive men in stage I and controls His group in stage I consisted of 12 men of whom 8 were under 40 years There were not sufficient men of various ages in stage I to show whether the absence of increase in the TPRI was only characteristic in younger subjects The present study demonstrates that in men in stage I over 30 years, the TPRI is significantly higher than in controls Furthermore, the TPRI is significantly higher in hypertensives 40–49 years than in the youngest hypertensive group

The highest resistance values were found in men in stage II and III, consistent with observations by others (16, 46, 135, 158)

The statistical significance of the differences between the groups is not affected if the peripheral resistance is not corrected for BSA

g) Left ventricular work index (Table 17 and fig 16)

Normotensives The mean value of the total group is 4 19 kpm/min/m^2 and there is no clear tendency to changes with aging

Hypertensives The mean values in the three youngest hypertensive groups are highly significantly higher than in the controls In the oldest group the difference is insignificant The LVWI is significantly lower in the oldest hypertensive group than in the younger groups despite the MAP being highest in this group

Thus in the earlier phases of essential hypertension the heart is able to work

Table 17 *Left ventricular work index at rest sitting (kpm/min/m²) (Age groups number of subjects and legend as in table 12)*

Age group	Mean SD NT	HT
1	4 12	5 69***
	0 54	1 05
2	4 36	5 91***
	0 60	1 25
3	4 08	5 58***
	0 55	1 15
4		4 41
		1 17
		1–4*
		2–4**
		3–4*

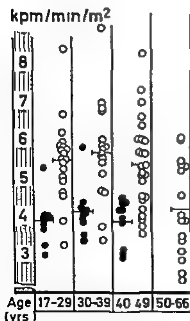


Fig 16 Left ventricular work index at rest sitting (Legend as in fig 8)

against the increased pressure and pump flow volumes so high that the flow pressure product is higher than in normals But in more advanced stages – (without *clinical* heart failure) –, the flow is so low that the work of the heart is no longer significantly *higher than in normals in spite of the high* pressure factor

b) Left ventricular stroke work index
(Table 18 and fig 17)

Normotensives The mean value of the total group is 62 1 pm/stroke/m and there is no clear tendency to changes with aging

Hypertensives The mean values in the 3 youngest hypertensive groups are higher than the values in the control groups The difference is significant or almost significant in age groups 1 and 3 In the oldest group, the mean value is slightly lower than in the control group The LVSWI in the oldest hypertensive group is almost significantly lower than in the other hypertensive groups Thus the

Table 18 *Left ventricular stroke work index at rest, sitting (pm/stroke/m²) (Age groups number of subjects and legend as in table 12)*

Age group	Mean SD NT	HT
1	60 8	71 9**
	10 1	12 0
2	64 5	73 1
	11 3	11 6
3	60 9	73 2*
	11 4	14 0
4		59 3
		20 4
		1–4*
		2–4*
		3–4*

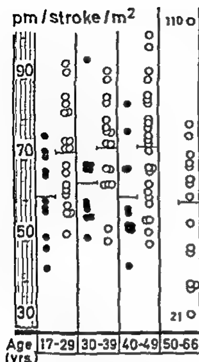


Fig 17 Left ventricular stroke work index at rest sitting (Legend as in fig 8)

stroke work of the heart is only significantly higher than in normals in the earlier phases of essential hypertension In later phases the volume delivered per stroke is so low that the high pressure does not compensate for it, and the work of the heart is *less* than in controls and less than in earlier hypertension This could be interpreted as an early sign of heart failure The heart no longer performs the extra work load superimposed by the high pressure

i) Arteriovenous difference (Table 19 and fig 18)

Normotensives The mean value in the total normotensive group is 43 5 ml/l very similar to the value found in Sannerstedt s (135) controls (45 5 ml/l) These values compare well with those obtained in the supine position in 19 studies summarized by Muller (118), if it is taken into account that the CO is lower and the VO_2 higher when erect or sitting than when supine (29, 130)

Hypertensives The *two oldest* hypertensive groups have *higher* mean values than their control group, the difference being highly significant in the oldest group In the *two youngest groups* the mean values are practically identical with the control values The value tends to increase with aging and is highly significantly higher in the oldest group than in any of the other age groups Similar results were found by Sannerstedt (135) and Taylor *et al* (155) It is probable that the increased arteriovenous difference in the subjects over 40 years represents an early sign of a reduced cardiac reserve

Since the arteriovenous difference is normal and not reduced in the youngest hypertensives (only two subjects have rather low values), they have no "luxury per

Table 19 *Arteriovenous difference at rest, sitting (ml/l) (Age groups number of subjects and legend as in table 11)*

Age group	Mean SD NT	Mean SD HT
1	45 0 7 1	44 6 7 3
2	42 2 6 2	42 4 6 7
3	43 2 5 1	49 7** 8 9
4		62 2*** 12 9
		1–4*** 2–4*** 3–4***

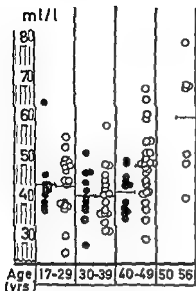


Fig 18 Arteriovenous oxygen difference at rest sitting (Legend as in fig 8)

fusion The condition is therefore different from the hyperkinetic syndrome described by Gorlin (66) and Snyder (147) Gorlin found a mean arteriovenous difference of 28 ml/l in his group of hyperkinetic subjects Systolic hypertension was frequent in their patients and Snyder states that it is conceivable that these patients represent a prehypertensive clinical category found in certain subjects who later develop essential hypertension

2 Supine position

The supine group is small, and is included mainly in order to give a complete survey of the hemodynamics at rest in *all* subjects studied in this investigation (page 15)

Since the number of subjects is so small, those under 40 years are included in one group and the statistical calculations are omitted (all controls are < 40 years)

a) Cardiac index (Fig 19)
In the normotensive group the mean value is 3 45 l/min/m² (0 10 l/min/m higher than the mean value for the total normotensive sitting group)

The mean values in the hypertensive groups < 40 40-49, 50-61 years are 3 61 3 66 and 3 18 l/min/m² respectively There is thus a tendency for lower CI in hypertensives over 50 years than under 50 years

b) Heart rate (Fig 20)
In the normotensives the mean value is 63 3 (against 67 9 for the total sitting normotensive group) The mean values in the

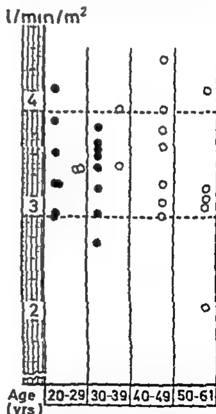


Fig 19 Cardiac index at rest supine (Legend as in fig 8)

cently Ward *et al.* 1966 (161) reported a considerably smaller drop in transition from supine to chair position — 9.8% (0.7 l/min).

As stated, the results in the *hypertensives* show the same tendencies as those obtained in the sitting group.

3. Survey of the flow-resistance pattern at rest.

Since the hemodynamics in the sitting and the supine positions are different, the results obtained in the different body positions cannot be compared directly. In order to be able to show the hemodynamic pattern in *all* subjects studied, the flow and resistance values were divided into groups separated by arbitrary borderlines so that about 75% of the values for flow and resistance *in the normotensives fell within* a class called "medium". Values above or below this range were termed "high" or "low".

The purpose of this classification was to see whether any trend in the flow-resistance pattern not demonstrable by the statistical evaluation in the sitting position alone, could be detected.

The ranges for the "medium" class for flow (CI) are 2.8–3.6 l/min/m² sitting and 3.0–4.0 l/min/m² supine, and for the resistance (TPRI) 2000–2700 dyn sec. cm⁻⁵m² sitting and 1700–2350 dyn sec. cm⁻⁵m² supine. The borderlines are shown in figs. 9, 15, 19 and 23.

Table 20 and fig. 24 demonstrate the flow resistance pattern after this classification.

It is seen that in the *normotensive* group, the majority, approximately 75%, has by definition a medium flow and medium resistance irrespective of age. A few have a high flow but, since the resistance is low, they have a normal blood pressure. In all age groups one or two have a low output and medium resistance and one in the oldest group has high resistance.

In the *hypertensive* group the hemodynamic pattern is *clearly different in the different age groups*. In the youngest group, the majority, 67%, have high flow and medium

Table 20. *Hemodynamic pattern in 141 males at rest*

CI		High			Medium			Low		
TPRI		Low	Medium	High	Low	Medium	High	Low	Medium	High
Normotensives										
Age (years)	n									
17–29	18	1	1		1	14			1	
30–39	19	3			1	14			1	
40–49	11		1			8			1	1
Hypertensives										
17–29	21		14			4	1			2
30–39	19		8	1		3	5			2
40–49	32		2	6		1	18			5
50–66	21		1				7			13

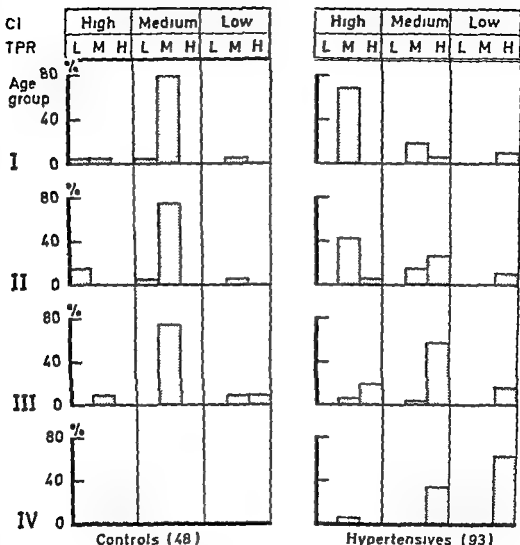


Fig 24 The hemodynamic pattern at rest in all subjects studied. (L = low M = medium H = high)

resistance Some have borderline values of both flow and resistance, and fall in the medium flow medium resistance group and a few 10 % have low output and high resistance Altogether only 14 % have high resistance In the age group 30-39 years the individuals are more scattered but 47 % have high output and 5 % also have high resistance The rest are scattered in some of the other groups as shown in the

cently, Ward *et al* 1966 (161) reported a considerably smaller drop in transition from supine to chair position — 9 8 % (0 7 l/min)

As stated the results in the *hypertensives* show the same tendencies as those obtained in the sitting group

3 Survey of the flow-resistance pattern at rest.

Since the hemodynamics in the sitting and the supine positions are different, the results obtained in the different body positions cannot be compared directly In order to be able to show the hemodynamic pattern in *all* subjects studied, the flow and resistance values were divided into groups separated by arbitrary borderlines so that about 75 % of the values for flow and resistance in the normotensives fell within a class called ' medium Values above or below this range were termed high or low

The purpose of this classification was to see whether any trend in the flow resistance pattern not demonstrable by the statistical evaluation in the sitting position alone, could be detected

The ranges for the "medium class for flow (CI) are 2 8–3 6 l/min/m² sitting and 3 0–4 0 l/min/m² supine, and for the resistance (TPRI) 2000–2700 dyn sec cm⁻⁵m² sitting and 1700–2350 dyn sec cm⁻⁵m² supine The borderlines are shown in figs 9, 15, 19 and 23

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It is seen that in the *normotensive* group, the majority, approximately 75 %, has by definition a medium flow and medium resistance irrespective of age A few have a high flow but, since the resistance is low, they have a normal blood pressure In all age groups one or two have a low output and medium resistance, and one in the oldest group has high resistance

In the *hypertensive* group the hemodynamic pattern is *clearly different in the different age groups* In the youngest group the majority 67 % have high flow and medium

Table 20 *Hemodynamic pattern in 141 males at rest*

CI		High			Medium			Low		
TPRI		Low	Medium	High	Low	Medium	High	Low	Medium	High
Normotensives										
Age (years)	n									
17–29	18	1	1		1	*14*			1	
30–39	19	3			1	*14*			1	
40–49	11		1			*8*			1	1
Hypertensives										
17–29	21		*14*			4	1			[illegible]
30–39	19		*8*	1		3	5			[illegible]
40–49	32		2	[illegible]		1	*18*			[illegible]
50–66	21		1				7			*13*

Table 22 *Variations in the peripheral resistance determined 30–90 min after catheterization. Legend as in table 21 (dyn.sec cm⁻⁵m²)*

Normotensives (5)				Hypertensives (7)			
I	II	III	III–II	I	II	III	III–II
1753	1861	2040	+ 179 (+ 9 6)	1862	1970	2000	+ 30 (+ 1 5)
2005	2201	2162	− 39 (− 1 8)	2611	2437	2468	+ 31 (+ 1 3)
–	1924	1982	+ 58 (+ 3 0)	2243	2273	2173	− 100 (− 4 4)
–	1865	2143	+ 278 (+ 14 9)	–	2980	2904	− 76 (− 2 6)
1623	1613	1604	− 9 (− 0 6)	2285	2421	2305	− 116 (− 4 8)
				3439	3134	3092	− 42 (− 1 3)
				4950	5220	5270	+ 50 (+ 1 0)
Mean –	1893	1986	+ 93 (+ 4 9)	–	2919	2887	− 32 (− 1 1)

within + 3 5 % to − 6 2 %, in both hypertensives and controls The mean changes were only −2 5 % and −0 4 % in the groups respectively The total peripheral resistance was also quite stable from the 60th to the 90th minute, particularly in the hypertensives where the individual changes were within + 1 5 % to −4 8 % The mean changes in the controls and hypertensives respectively were +4 9 % and −1 1 % The stability is dependent upon a quiet atmosphere in the laboratory and no manipulations with the subject but – on the other hand the subject must not fall asleep This is evident from the following observations

a2) Effect of external measurement of the blood pressure

In 35 hypertensive subjects in whom the BP was fairly stable 20–30 minutes after catheterization the investigator measured the blood pressure 3 times at intervals of a few seconds by the conventional cuff method while the intraarterial blood pressure was recorded continuously on the opposite arm The subjects were not informed that this was going to take place

Table 23 *The changes in the intraarterial brachial artery pressure induced by recording the blood pressure externally on the opposite arm (mm Hg – numbers in parentheses are per cent change)*

Age years	n	SAP			DAP		
		Before	Change during recording		Before	Change during recording	
		Mean	Range	Mean	Mean	Range	Mean
<40	13	142 4	0 − +16	+ 6 3(+4 4)	81 1	0 − +11	+4.5(+5 5)
40–49	17	150 6	+3 − +17	+10 6(+7 0)	87 4	+1 − +11	+5 7(+6 5)
50–56	5	175 2	+3 − +14	+ 8 0(+4 6)	92 6	0 − +25	+7 8(+8 4)
Total	35	151 1	0 − +17	+8 6 (+5 7)	85 8	0 − +25	+5 5(+6 4)

table Altogether 42% have high resistance In the age group 40–49 years, 25% have high output but the majority of these have high resistance Most of the group, 56 %, [illegible] found with the pattern medium output and high resistance, and altogether 91% have high resistance

In the oldest group, 62% have low output and high resistance and 33% medium flow and high resistance, the same pattern as is characteristic for the preceeding decade Only one individual over 50 years has the pattern characteristic for the juvenile group and altogether, 95% have high resistance

Thus, in the hypertensive men studied, there is a clear trend to a *change* in the flow resistance pattern, from a "high flow normal resistance" pattern in the subjects under 30 years to a 'low flow high resistance' pattern in subjects over 50 years of age, most of whom had complications

It is possible that the "high flow high resistance pattern seen in the age group 30–50 years, represents a transitory stage before the medium flow high resistance" pattern which dominates in the age group 40–49 years

4 Variations in the hemodynamics at rest, supine

a) Short time observations

It would be of interest to know whether or not the hemodynamic pattern changes during prolonged rest, and how easily the flow, pressure and resistance could be influenced by external disturbances Knowledge of such variations [illegible] also necessary for the interpretation of the effect of dextran infusion and of a heavy meal

a1) Undisturbed subjects

Five normotensives and 7 hypertensives rested comfortably in a quiet laboratory but were not allowed to sleep Double determinations of the cardiac output and the pressure were performed 30, 60 and 90 minutes after the catheterization and the TPRI was calculated (Owing to technical problems, the observations after 30 minutes were not obtained in 3 subjects)

The results are shown in table 21 and 22 Between the 30th and the 60th minute, the CI sometimes dropped more than 10% But from the 60th to the 90th minute the individual variations in the CI were

Table 21 *Variations in the cardiac index determined 30–90 min after catheterization (mean of 2 measurements) (I = 30 min II = 60 min III = 90 min The numbers in parentheses are III–II as per cent of II)* *(l/min/m²)*

Normotensives (5)				Hypertensives (7)			
I	II	III	III–II	I	II	III	III–II
3 97	3 61	3 45	−0 16 (−4 4)	4 51	4 02	4 04	+0 02 (+0 5)
3 67	3 27	3 33	+0 06 (+1 8)	4 38	4 53	4 31	−0 22 (−4 9)
–	3 70	3 47	−0 23 (−6 2)	3 78	3 73	3 68	−0 05 (−1 3)
–	3 86	3 62	−0 24 (−6 2)	—	3 14	3 14	[illegible] (0)
3 40	3 47	3 59	+0 12 (+3 5)	4 27	4 03	4 13	+0 10 (+2 5)
				3 14	3 19	3 26	+0 07 (+2 2)
				2 23	2 13	2 11	−0 02 (−0 9)
Mean —	3 582	3 492	−0 090(−2 5)	—	3 539	3 524	−0 015(−0 4)

tients in connection with renal arteriography 30 minutes after the arteriography was completed The renal artery catheter was used for the withdrawal curve Although the experimental situation was different in the second study it should give an indication as to whether or not the peripheral resistance was mainly the same as in the first study

The results demonstrated that in all but one individual the resistance pattern was unchanged but there were some changes in the flow pattern The one individual who had changed his resistance pattern had higher resistance than in the first study The results are found in the individual data table[1]

The resistance pattern in resting subjects seems to be quite characteristic for the individual

This is consistent with the results reported by Eich *et al* (41 42)

There is a great difference in the hemodynamics in two hypertensives with the same systemic arterial pressure where one has a high flow and a normal resistance – the other a subnormal flow and very high resistance As others have also pointed out (94) the hemodynamic pattern should be clarified in order to achieve a rational antihypertensive therapy It is also likely that it would give a better indication of the severity of the disease than measurement of the BP alone (46 94)

B Studies during exercise

1 Results.

When the findings in the age groups are described the sequence is always < 30 30–39 40–49 and > 50 years Since controls over 50 years were not studied the results in the 7 men 50–56 years old are compared with normotensives aged 40–49 years This of course demands great care in the interpretation of these results

a) Oxygen consumption. Table 25)

Normotensives In relation to the resting values (table 11) the VO at the 900 kpm load increased 1045 8–1054 5 and 1150.5 ml/min or 713–707 and 825% in the three groups The VO during exercise did

Table 25 *The oxygen uptake during work [illegible]*

Age group years	n NT	n HT	300 kpm/min Mean SD NT	300 kpm/min Mean SD HT	600 kpm/min Mean SD NT	600 kpm/min Mean SD HT	900 kpm/min Mean SD NT	900 kpm/min Mean SD HT
1 17–29	11	19	465 5	499 1	[illegible]	810 4	1192 5	1254.2
			45 8	64 6	56	55 4	118 1	117 9
2 30–39	11	17	453 0	486.5	[illegible]	754.5	1180 8	1149 1
			39 7	64 4	62.2	8 [illegible] 1	117.5	81 7
3 40–49	11	25	4 6 4	511 3	840 0	835 4	1289 9	1 03 6
			43 1	4 0	91.2	83 8	[illegible]	173.4
4 50–56	0	7		484 6		769 9		1162 8
				9 4		16 8		58

[1] Subjects no 4 5 13 16 18 25 38 55 main table part B Subjects no 8 33 54 51 53, 59 65 main table part C.

Table 26 *The oxygen uptake in relation to work load in non athletic Scandinavian men below 50 years (l/min)*

Author and year	Subjects Occupation	n	Work Load (kpm/min)		
			300	600	900
Asmussen and Nielsen, 1953	Students	25	910	1429*	2143
Grimby, 1962	*Various*	*38*	*880*	*1480*	*2270*
Hermansen, 1964	Office workers	40	—	1577	2150
Lund Johansen, 1967	Various	33	891	1516	2341

* Work load 540 kpm/min

not show any significant changes with aging (table 25) although the values tend to be higher in the oldest group probably due to reduced cycling efficiency The results compare well with similar studies from other Scandinavian laboratories (8, 72, 85) as is evident from table 26, (note that the values are not corrected for BSA) No subjects had real difficulty in performing the 900 kpm/min load for 7–8 minutes – in contrast to recent reports from USA (99) where unselected subjects aged 35–49 years were reported to be exhausted at a VO_2 of 1550 ml/min A recent report from Canada (14) also states that unselected Canadian volunteers seem to be less fit than Scandinavians

Hypertensives During light work (300 kpm/min), the VO_2 tends to be higher in the hypertensives, as at rest At the highest work load, the VO_2 in the three oldest groups is lower than in the controls *It is not impossible that the maximal* VO_2 had been reached in some of these patients and that they had a lower maximal VO_2 than the VO_2 at 900 kpm load in the controls The values are close to the maximal VO_2 in Scandinavian men, about 50 years old, in manual work (167)

In relation to the resting values the absolute increase of VO_2 in the four age groups at the 900 kpm load is about as much as in the controls (1090 6 – 1000 0– 1048 8 and 1015 5 ml/min/m²), but the relative increase is somewhat less (667 – 671 – 678 and 662%)

The differences from the control group however, are not significant This makes the calculations of the difference between the hemodynamic parameters in the hypertensives and controls more simple It seems justified to calculate the differences between the groups at each work level (instead of relating all values to oxygen uptake as is done in the figures) However the relationship between the blood flow (CO) and the oxygen uptake will be analysed separately (in addition to the analyses of the cardiac index at the 3 work loads)

b) Cardiac output (Table 27 and fig 26) There is considerable scatter in the individual values During work there is an almost linear relationship between the CI and oxygen uptake in all groups

However in transition from rest to light exercise, the curve is steeper than from light to heavy work and, as seen from table 28, the blood flow per ml O_2 uptake decreases with increasing load in all groups

Table 27 *The cardiac index during work (l/min/m²) (Age groups and number of subjects as in table 25. The asterisks in the table show significance of the difference between hypertensives and controls. The significance of the differences between the age groups is marked below the table)*

Age group	300 kpm/min Mean SD NT	300 kpm/min HT	600 kpm/min Mean SD NT	600 kpm/min HT	900 kpm/min. Mean SD NT	900 kpm/min. HT
1	7 14	6 90	9 22	9 10	11 89	11.21
	0 91	1 05	1.26	0 74	0 73	0 94
2	7 11	6 82	9 94	8 66**	12.43	10 41***
	0 75	0 84	0 89	1 08	0 96	1 27
3	6 48	6 30	9 17	8 23*	11 57	9 90***
	0 82	0 68	1 01	0 94	1 06	0 68
4		5 54*		6 93***		8 84***
		0 74		1 41		1 56
		(3-4*)		1-3* (3-4*)		1-3** (3-4*)

Normotensives The cardiac index increases with increasing work load with little difference in the three age groups studied Thus at 900 kpm/min the ranges in the three groups are 12 97 – 10 67, 13 94 – 10 47 and 13 40 – 10 22 l/min/m respectively and the mean values 11 89 12 43 and 11 57 l/min/m². The increases in the mean values from rest (table 12) to 900 kpm load are 8 60 8 92 and 8 32 l/min/m² respectively or 261 – 254 and 256 %. Thus there is no significant effect of aging from 20 to 50 years (confirmed by the statistical analyses)

Several workers (38 56 80 99) have reported no significant decrease in CO in relation to work load (or oxygen uptake) in normal men with aging up to 50 years Recently Becklake *et al* (14) reported that the cardiac output in relation to work load was higher in older than in younger subjects (Canadians) In the present study the cardiac output is higher in relation to work load and oxygen uptake than reported by most workers (99 135, 136, 159) This is partly due to body position and probably also to a high habitual physical activity The values are about 10 –15 % higher than those reported by Asmussen & Nielsen (8) who also used an armchair ergometer bicycle (Danes) The values at 900 kpm/min load are about 50 % higher than the maximal values in an untrained group of American volunteers (99) on an ordinary saddle seat ergometer bicycle

Hypertensives At all work levels and in all age groups including the youngest the cardiac index tends to be lower than in the controls The difference between the hypertensives and the controls increases with increasing age In the youngest group the difference is insignificant at all work levels The difference is significant or almost significant in age groups 30–39 and 40–49 years at the two heaviest loads and in the oldest group at all loads While there was no significant effect of aging upon the CI during work in the normotensives the CI in the hyper

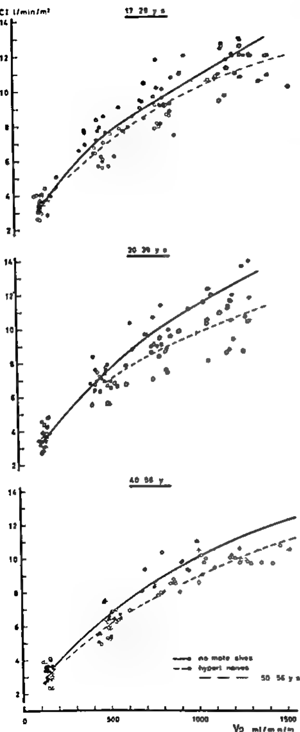


Fig 26

Fig 26 The cardiac index in relation to oxygen uptake at rest and during work The curves are drawn through the mean values for CI and VO_2 at rest and at 300, 600 and 900 kpm/ loads The vertical dotted lines are drawn through 1000 ml/min/m², the horizontal lines through CI = 10 5 l/min/m²

tensives decreases with increasing age as is evident from table 27 At the two highest work loads, the CI is significantly or al most significantly lower in the age group 40–49 years than in the youngest group and almost significantly lower in hyper tensives > 50 years than in hypertensives aged 40–49 years The relative increase in the CI from rest (table 12) to the highest work load is 202–191–211 and 259% in the four age groups The *relative* increase is thus *highest* in the oldest age group but this is due to the low resting output in this group The *absolute* increases are 7 50 – 6 83 – 6 72 and 6 38 l/min/m , thus clearly lower than in the controls and lowest in the oldest group

With regard to individual values at the highest work level, 5 of 18 hypertensives in the youngest group have values below the lowest value in the control group (10 67 l/min/m) In the age group 30–39 years 7 of 16 have values lower than the lowest value in the controls (10 47 l/min/m), and in the age group 40–49 years, 19 of 24 have lower values than the lowest value in the control group (10 22 l/min/m²)

Only 4 in the oldest group were studied at this level, and 3 of them have values < 8 6 l/min/m²

It is interesting that already in transition from rest to light work (300 kpm/min) the hemodynamic pattern in the youngest group changed from flow higher than in the controls to "flow lower than in the controls"

Table 28 *The cardiac output in relation to oxygen uptake (ml/ml O_2 per min)* (*Legend as in table 27*)

Age group	Rest Mean SD NT	Rest HT	300 kpm/min Mean SD NT	300 kpm/min HT	600 kpm/min Mean SD NT	600 kpm/min HT	900 kpm/min Mean SD NT	900 kpm/min HT
1	22.6	23.1	15.4	14.0	11.9	11.2	10.0	9.0*
	2.7	4.5	1.9	2.8	1.6	1.3	1.2	1.1
2	24.2	24.1	15.7	13.8*	13.0	11.1**	10.5	9.1**
	3.9	3.6	2.1	1.8	1.8	1.1	1.2	1.4
3	23.4	20.7	13.7	12.4*	11.2	9.8**	9.0	8.3
	3.3	4.0	1.9	1.5	1.3	1.3	1.2	1.2
4		16.8***		11.4*		8.7**		7.6
		3.8		1.5		1.8		1.9
		1–4*** 2–4***	2–3*	1–3*	2–3*	1–3** 2–3**	2–3**	

If we look at the blood flow in relation to the oxygen uptake (table 28) it is seen that in the *youngest* group, the flow per ml O_2 uptake per min *at rest* is slightly *higher* than in the controls, but during all work loads it is *lower*. At the 900 kpm/min load the difference is almost significant. In the age group 30–39 years, the *resting* values are almost identical in the normotensives and hypertensives but, during work the values are significantly lower in the hypertensives. In the age group 40–49 years the values are lower in the hypertensives than in the normotensives, *both* at rest and during work, the difference being significant or almost significant at the 300 and 600 kpm load. In the oldest hypertensive group, the flow at rest is highly significantly lower than in the controls and also lower than in the controls during work.

Thus, if we regard the blood flow as CI in relation to the work load or as ml blood flow per ml O_2 uptake per min, we find that the flow during work is clearly lower in hypertensives than in controls in all age groups, and is lowest in the oldest. This means that if the oxygen need in the working muscles is the same in the hypertensives as in the controls the arteriovenous oxygen difference must be increased in the hypertensives in order to maintain aerobic metabolism.

Sannerstedt (135) also found that the cardiac output rose less with the increase in oxygen consumption in hypertensive men and women in stage I, but found that the subjects in stages II and III did *not* differ from the controls in this respect. This is in contrast to the present findings where the lowest blood flow during work was found in the oldest hypertensive group with some subjects in stage II and III. There is a clear tendency to a lower blood flow in relation to oxygen uptake with increasing age in the hypertensives.

Amery *et al.* (2) found that the maximal CI was reduced in young hypertensives but in older groups the difference from the controls was not significant. The discordance is probably due to different selection of patients.

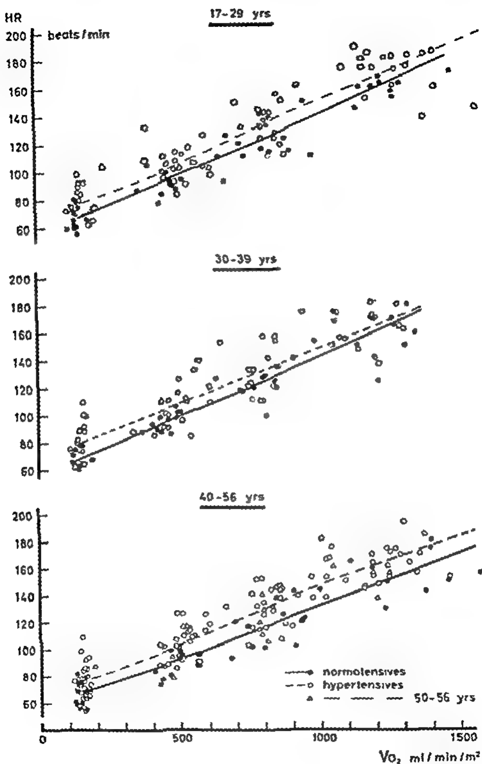


Fig 27 The heart rate in relation to oxygen uptake at rest and during work. The curves are drawn through the mean VO_2 and HR at rest and at the 3 work loads ■ represents a well trained marathon runner

Table 29 *The heart rate during work (beats/min) (Legend as in table 27)*

Age group	300 kpm/min Mean SD		600 kpm/min Mean SD		900 kpm/min Mean SD	
	NT	HT	NT	HT	NT	HT
1	95.0	105.0**	122.5	135.8*	160.2	171.0*
	8.2	11.4	10.3	15.0	9.5	14.4
2	94.8	108.3*	122.1	132.6	160.3	161.1
	6.8	19.4	9.0	19.7	14.8	14.0
3	90.6	105.2**	118.1	134.4**	155.1	162.5
	10.1	12.0	14.2	15.9	14.4	14.3
4		96.1		122.1		152.3
		10.7		11.7		9.6

c) Heart rate *(Table 29 and fig 27)* The well known linear relationship between heart rate and VO_2 in normals (8, 9, 72, 85) is demonstrated. The same relationship is also seen in the hypertensives.

Normotensives. The heart rate during work tends to decrease with increasing age but the differences between the groups are not significant. The mean values at the three work loads agree well with those reported from other Scandinavian laboratories (8, 72, 85).

The heart rate in relation to oxygen uptake is lower than in untrained American and Canadian volunteers during bicycling in the sitting position (14, 99).

Hypertensives. In the 3 youngest groups, the heart rate is higher than in the controls, most marked during light work. In the oldest group, the heart rate is slightly lower than in the control group at the highest work level. The increase in heart rate from rest (table 13) to the highest work load is 91.9 (116%), 80.3 (99%), 85.8 (111%) and 75.2 (98%) in the four hypertensive groups against 92.1 (135%), 92.5 (136%), 87.4 (129%) in the three control groups.

Thus, the *absolute* increase in the heart rate is largely the same in the hypertensives as in the controls in the three youngest groups, consistent with the findings of Sannerstedt (135) and Konig *et al* (105).

The lower cardiac index in the hypertensives during work is thus *not* due to a low frequency of the heart, and must consequently be due to a reduced stroke volume.

d) Stroke index (Table 30 and fig 28) There is a considerable scatter in the individual values. Fig 28 demonstrates that, in transition from rest to exercise, there is an increase in the SI in all age groups but, after the initial increase which appeared already at the 300 kpm load, the SI remained rather constant. This is consistent with the findings of Chapman et al (32) and Wang *et al* (160).

Normotensives. The stroke index during work is not significantly influenced by age. The increase from rest (table 14) to heavy work is 25.5 (52.2%), 26.1 (50%) and 26.4 ml/stroke/m² (54%) in the three age groups.

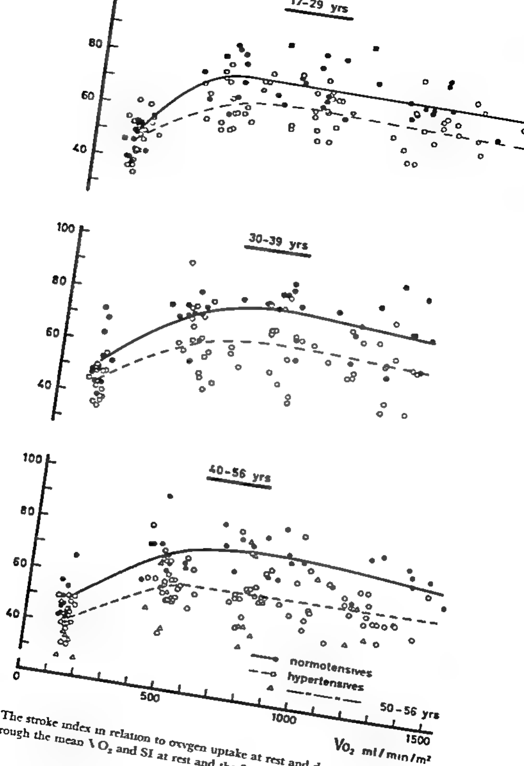


Fig 28 The stroke index in relation to oxygen uptake at rest and during work The curves are drawn through the mean $\dot{V}O_2$ and SI at rest and the 3 work loads ■ represents a well trained marathon runner

Table 30 *The stroke index during work (ml/stroke/m²) (Legend as in table 27)*

Age group	300 kpm/min Mean SD		600 kpm/min Mean SD		900 kpm/min Mean SD	
	NT	HT	NT	HT	NT	HT
1	75 3	65 8**	75.2	67 6*	74 4	66 0**
	8 1	8 0	7 5	7 5	5 6	8 0
2	75 2	63 4**	81 7	66 4***	78 4	65 1**
	8 9	10 0	7 8	11 1	10 9	10 2–
3	71 8	60 5***	78 1	61 7***	74 9	61 6***
	8 2	8 0	6 7	7 9	6 2	8 2
4		58 1**		57 7***		58 1***
		8 8		14 9		9 4

(A well trained marathon runner studied in this laboratory increased his SV by almost 100%)

The SI values are higher than those reported by others (56, 99 135, 154, 168) This is partly due to body position and probably partly to a relatively good physical condition in the subjects since a high SV has been shown to be characteristic in trained subjects (60, 73)

Hypertensives The stroke index is lower than in the controls at all work loads in all age groups and, with one exception, all differences are significant or highly significant It is seen from table 30 that the stroke index decreases with increasing age – in contrast to what is found in the controls However, the differences between the age groups 1–3 are not significant The increase in the stroke index from rest (table 14) to the highest work load in the four age groups is 18 68 (39%) – 20 43 (46%) – 19 60 (47%) and 25 12 ml/stroke/m² (76%) The absolute increase is less than in the controls in the three youngest groups and about the same as in the controls in the oldest group Thus the *increase* was least in the youngest group and greatest in the oldest The actual stroke volume delivered by the heart during exercise dropped with increasing age

As the body position of the subject is of such great importance for the stroke volume response during work, the present results are difficult to compare with others obtained supine or sitting more vertically Sannerstedt (135) found that the stroke volume tended to *drop* in hypertensives in stage I in transition from rest (sitting in a chair) to bicycling (vertically) whereas the stroke volume rose in the control and in the hypertensives in stage II and III In the present study the subjects sat in the same body position at rest as during work (except for the legs off or on the pedals) All subjects showed a rise in stroke volume under these circumstances but as mentioned, the *rise* was less (absolutely and relatively) in the youngest hypertensives, However it must be emphasized that the small increase was due to a relatively high resting stroke volume – the actual SV during work was higher in this than in the other hypertensive groups

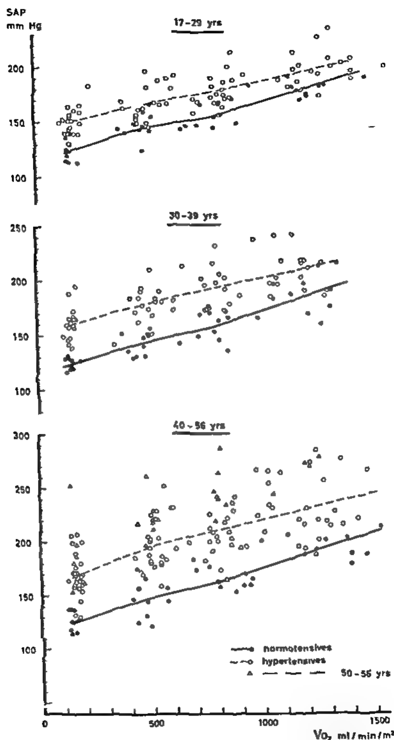


Fig 29 The systolic arterial pressure at rest and during work The curves are drawn through the mean VO_2 and SAP at rest and at the ▌ work loads

Table 31 *Systolic arterial pressure during work (mm Hg)* (*Legend as in table 27*)

Age group	300 kpm/min Mean SD NT	HT	600 kpm/min Mean SD NT	HT	900 kpm/min. Mean SD NT	HT
1	143 1	166 4***	153 5	175 9***	178 1	194 9**
	12 9	15 2	9 7	13 9	6 1	15 0
2	142 1	178 4***	156.2	191 6***	183 2	205 7**
	9 7	17 6	13 3	20 5	17 3	17 1
3	147 1	197 4***	164 5	212 3***	194 9	229 5***
	16 6	23 1	9 3	25 8	10 2	27 1
4		224 4***		246 6***		264 8***
		18 8		20 4		13 0
		1–3***		1–3***	1–3*	1–3***
		2–3***		2–3**		2–3**
		(3–4**)		(3–4**)		(3–4*)

e) Blood pressure

e1) *Systolic blood pressure* (Table 31 and fig 29)

Normotensives The SAP increases more with increasing load in the oldest group than in the youngest. The rise in the SAP from rest (table 15) to the highest work load is 55 3 mm Hg (45 %) 59 7 mm Hg (48 %) and 69 9 mm Hg (56 %) in the three age groups Thus there is a clear tendency for the SAP to increase with increasing age and while there is no significant difference between the SAP in the three groups at rest, the SAP at the 900 kpm load is almost significantly higher in the oldest group than in the youngest.

Hypertensives During all work loads the SAP is significantly or highly significantly higher than in the controls in all age groups The rise in the SAP from rest (table 15) to the highest work load is 44 7 mm Hg (30 %) 45 8 mm Hg (29 %) 60 7 mm Hg (36 %) and 80 5 mm Hg (44 %) Thus the SAP rose *less* in the hypertensives than in the controls both absolutely and relatively (except for the absolute rise in the oldest hypertensive group) As in the normotensives, the pressure rises more with increasing age

e2) *Diastolic blood pressure* (Table 32 and fig 30)

Normotensives The DAP rises slightly in transition from rest to light or moderate exercise (600 kpm), and more in transition from the 600 to the 900 kpm load The rise in the DAP from rest to the 900 kpm load is 11 0 mm Hg (14 %), 18 0 mm Hg (25 %) and 20 4 mm Hg (27 %) in the three age groups Thus the DAP rises absolutely and relatively less than the SAP Like the SAP, the DAP rises more with increasing age and more markedly The rise in the oldest group is nearly twice as high as in the youngest and at the 900 kpm load, the DAP is almost significantly higher than in the youngest

Hypertensives The DAP is significantly or highly significantly higher in the hypertensives than in the controls in all age groups and at all work levels The

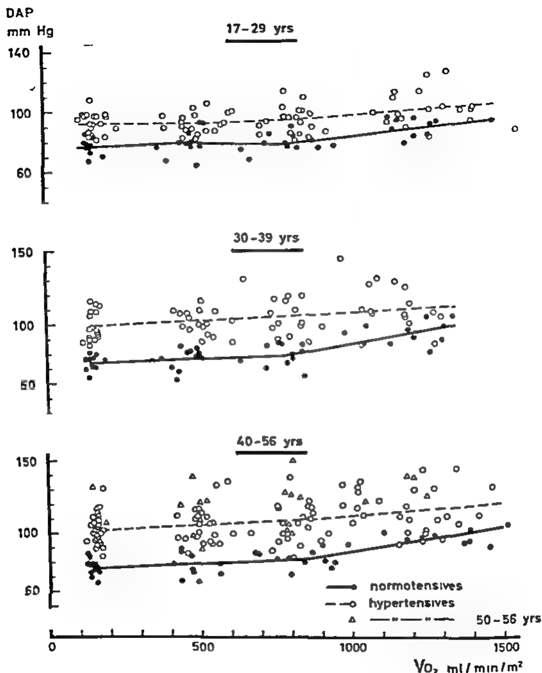


Fig 30 The diastolic arterial pressure at rest and during work Legend as in fig 29

Table 32 *Diastolic arterial pressure during work (mm Hg) (Legend as in table 27)*

Age group	300 kpm/min Mean SD NT	300 kpm/min Mean SD HT	600 kpm/min Mean SD NT	600 kpm/min Mean SD HT	900 kpm/min Mean SD NT	900 kpm/min Mean SD HT
1	78 5	91 8***	78 1	93 5***	87 5	100 8**
	8 4	6 6	5 6	8 8	6 5	11 3
2	76 4	101 8***	78 2	104 8***	93 1	108 6**
	6 8	11 9	7 2	14 2	7 4	13 5
3	78 1	106 7***	82 2	108 6***	96 5	114 8***
	7 8	12 4	5 4	14 2	4 5	14 5
4		113 7***		119 7***		131 0***
		15 7		16 7		7 6
		1–2** 1–3***		1–2** 1–3***	1–3*	1–3* (3–4*)

rise in the DAP from rest to the 900 kpm load is 8 4 mm Hg (9%) 9 6 mm Hg (10%), 13 2 mm Hg (13%) and 27 1 mm Hg (26 1%) in the four age groups Thus the DAP rises less in the hypertensives than in the controls both absolutely and relatively (except for the absolute rise in the oldest hypertensive group)

As in the controls the DAP rises more with increasing age

e3) *Mean arterial pressure* (Table 33 and fig 31)

Normotensives The MAP rises progressively in transition from rest to exercise The rise in the MAP from rest to the 900 kpm load is 24 5 mm Hg (26 6%) 31 mm Hg (33 9%) and 39 7 mm Hg (42 9%) in the three age groups Thus the rise in the MAP during heavy work increases with increasing age and while there is no difference between the MAP in the three age groups at rest the MAP at the 900 kpm load in the oldest group is significantly higher than in the youngest and almost significantly higher than in the group 30–39 years

Hypertensive During all work loads the MAP is significantly or highly significantly higher than in the controls in all age groups The rise in the MAP from rest to the 900 kpm load is 21 4 mm Hg (19%), 23 4 mm Hg (19 3%), 33 5 mm Hg (26 1%) and 54 9 mm Hg (41 9%) in the four age groups Thus the MAP rises less, absolutely and relatively in the hypertensives than in the controls (except for the absolute increase in the oldest hypertensive group) The rise in the MAP increases with increasing age

e4) *Comments on the pressure observations*

Already 40 years ago it was shown that the blood pressure response to exercise in hypertension differed from that in normotensives (12) and many studies have been carried out since then (see Sannerstedt (133) However, as determination of the arterial pressure by the cuff method is very inaccurate during work (100, 120) only findings obtained by the intraarterial method will be discussed Curiously enough there seem to be conflicting views upon the behaviour of the arterial blood

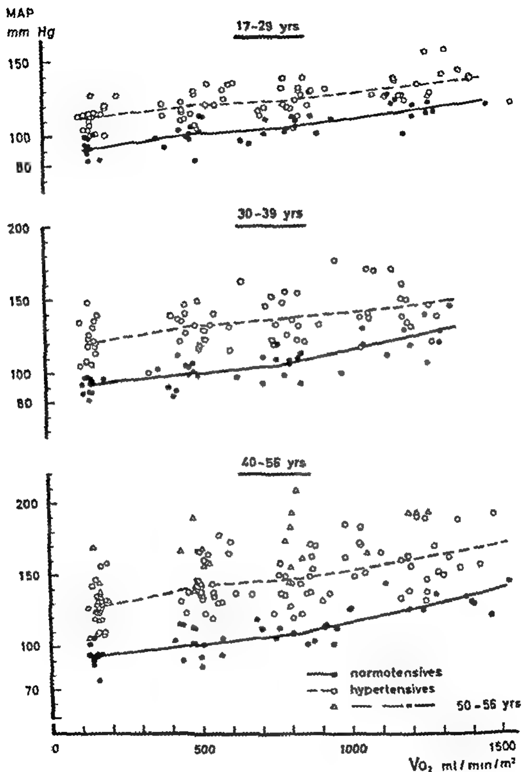


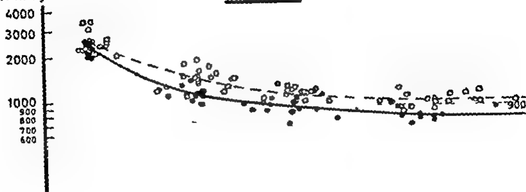
Fig 31 The mean arterial pressure at rest and during work Legend as in fig 29

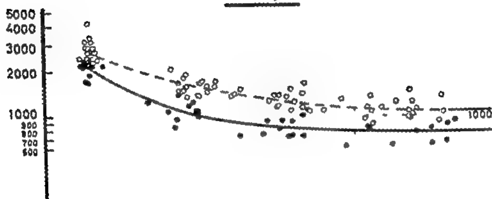
Table 33 *Mean arterial pressure during work (mm Hg) (Legend as in table 27)*

Age group	300 kpm/min Mean SD		600 kpm/min Mean SD		900 kpm/min Mean SD	
	NT	HT	NT	HT	NT	HT
1	100.2	120.2***	104.0	123.2***	116.4	134.1***
	8.1	9.2	6.0	8.8	6.9	10.1
2	98.7	132.1***	104.5	136.5***	122.5	144.3***
	8.0	13.9	8.4	15.9	12.8	15.8
3	102.2	143.4***	110.0	148.4***	132.2	161.8***
	9.7	16.1	5.5	18.5	7.5	17.4
4		158.7***		168.4***		186.0***
		17.0		18.6		12.1
		1–2*		1–2**		
		1–3***		1–3***	1–3**	1–3***
		2–3*		2–3*	2–3*	2–3***
		(3–4*)		(3–4*)		(3–4*)

pressure during muscular work in *normal man* Asmussen & Nielsen (9) have stated that there is a progressive rise in systolic and mean pressure with increasing exercise, and the present findings are in accordance with this view The rises in the present studies are greater during severe exercise than reported by Holmgren (91), who found an increase of 8 mm Hg systolic and 3 mm Hg in the mean arterial pressure per 300 kpm/min His group, however was one of well trained cyclists on a bicycle ergometer On the other hand, Fraser & Chapman (57) found that the intraarterially recorded systolic pressure rose and the diastolic *fell* and that the mean pressure showed no significant changes Recently Tabakin *et al* (154) reported a *fall* in the diastolic pressure during exercise in young men The last observation was made during treadmill walking and it is possible that the body position might be of importance for the blood pressure response In the supine position Grimby (72) found a rise in systolic diastolic and mean pressure during cycling so did Sannerstedt (135) and Julius *et al* (99) during cycling in the sitting position Extensive studies from Sweden (133) have shown that the upper normal limit for the blood pressure during cycling in the sitting position in men irrespective of age, is 219/110 mm Hg In the present study the highest normal values were 212 mm Hg systolic and 105 mm Hg diastolic which is similar to the Swedish results but the present study also clearly shows that the normal blood pressure response is dependent upon the age of the subject and increase with aging This is in accordance with several workers (69, 80 99) In the *hypertensives* too the brachial artery pressure rose more with increasing age consistent with reports by Amery *et al* (2)

When discussing the blood pressure response to exercise in hypertensive subjects it is necessary to distinguish between different types of patients From more recent work it is clear that the response is at least, dependent upon the age of the sub-





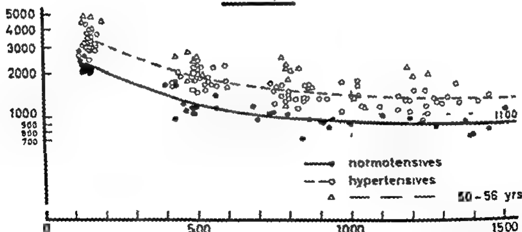


Fig 32 The total peripheral resistance index at rest and during work The vertical dotted lines are drawn through $\dot{V}O_2$ = 1000 ml/min/m² the horizontal lines as marked by the numbers

Table 34 *Total peripheral resistance index during work (dyn.sec cm⁻⁵m²)* (*Legend as in table 2*

Age group	300 kpm/min Mean SD		600 kpm/min Mean SD		900 kpm/min Mean SD	
	NT	HT	NT	HT	NT	
1	1132	1418***	915	1088***	786	9
	115	197	111	100	76	
2	1131	1590***	849	1271***	794	11
	196	183	116	160	112	
3	1287	1834***	970	1454***	921	13
	247	233	119	168	97	
4		2327***		2017***		17
		337		399		3.
		1–2*		1–2***	1–3**	1-
		1–3***		1–3***	2–3**	1-
		2–3***		2–3***		2-
		(3–4***)		(3–4***)		(3

ject and the stage of the hypertensive disorder Both Sannerstedt (135) and Amery *et al* (2) found no steeper rise in brachial arterial pressure in subjects who were in stage I (135) or < 35 years (2) than in controls In the present investigation the rise in the youngest hypertensive group was even somewhat lower than in the controls In older subjects with more severe hypertension the blood pressure rose more steeply in relation to oxygen uptake than in any of the other groups consistent with the findings by Sannerstedt (135) and Taylor *et al* (155) The observation that MAP in hypertensive men in stage I rises to the same extent or as in this study – a little *less* than in controls – could lead to the assumption that the peripheral vasodilation during work was even more effective than in the controls However the pressure responses must be seen in relation to the increase in blood flow and the problem will be discussed further in connection with the TPRI

f) Total peripheral resistance inde
(Table 34 and fig 32)

Both in normotensives and hyperte the TPRI drops with increasing most pronounced in transition from light exercise

Normotensives In transition rest (table 16) to the 300 kpm lo TPRI drops 50% in the youngest gr 44% in the oldest From light wo moderate work and from moderate to severe work the drop in resistance At 900 kpm load the TPRI is onl lower than at 600 kpm load The di the TPRI from rest to the 900 kpm l 1461 (65%) 1317 (62%) and 1378 sec cm⁻⁵m² (60%) in the three age gr Thus there is a small tendency fo TPRI to drop less with increasing age TPRI during the heaviest work lo significantly higher in age group years than in the two younger grou the individual values are studied it that at the highest work load onl

subject under 30 years has TPRI above 900 dyn sec $cm^{-5}m^2$, in the next decade 8 individuals have higher values, and in age group 40–49 years, 6 have higher values These findings are interesting in the light of the other hemodynamic parameters The *cardiac* parameters CI, HR and SI, showed no significant changes with increasing age during exercise but the resistance of the *vascular system* seems to be raised with aging from 20 to 50 years This is consistent with observations by others (80, 99)

It is difficult to compare the absolute values with those of previous investigations because of difference in body position In the supine position, Grimby (72) found higher values at 300 600 and 900 kpm/min and, during treadmill walking, Tabakin (154) found lower values at oxygen uptakes roughly similar to the three loads In untrained Americans, Julius *et al* (99) found considerably higher total peripheral resistance during maximal work (sitting) than in the present investigation at the 900 kpm load

Hypertensives During all work loads in all ages, the TPRI is significantly higher than in the controls The differences from the controls are usually greater during work than at rest At rest the TPRI is 10 7 – 30 8 – 42 8 and 86 5% higher than in the control groups During the 900 kpm/min load the corresponding figures are 22 1 – 41 1 – 42 7 – and 89 6% In the youngest group, the greatest difference between hypertensives and controls (+ 25 3%) is found at the 300 kpm/min load In this age group, there is no significant difference between the TPRI in hypertensives and controls at rest It is therefore particularly interesting that the TPRI during work is highly significantly higher than in the controls at all work loads, even in this youngest group The TPRI dropped from rest to 900 kpm load 1525 (61%), 1639 (59%), 1970 (60%) and 2268 dyn · sec $cm^{-5}m^2$ (60%) The relative drop in the TPRI is thus practically the same in all the hypertensive and normotensive groups! However, if we consider the individual values, it is seen that only 3 of 19 of the youngest hypertensives have peripheral resistance outside the normal range *at rest* but, at the 300 kpm/min load, 12 of 19 have higher values than the highest value in the control group (1375 dyn sec $cm^{-5}m^2$) At the highest work load, one normotensive exceeds 900 dyn sec cm^{-5} m , while 14 of the 18 hypertensives studied do so One of the three who had elevated resistance *at rest*, has resistance below 900 dyn sec $cm^{-5}m^2$ at the highest work load – the other two have higher values In the age group 30–39 years, only 7 of 17 hypertensives had values above the normal range *at rest* During 300 kpm/min 10 of 16 hypertensive exceed the highest control value (1521 dyn sec $cm^{-5}m^2$) At the same load, only 1 of the controls exceeds 1400 dyn sec $cm^{-5}m^2$, while all but two hypertensives do so At the 900 kpm/min load 13 of 16 hypertensives exceed the highest control value (985 dyn sec $cm^{-5}m^2$)

Thus, it is demonstrated that, during muscular exercise, the total peripheral resistance index in hypertensives, including those under 40 years, is usually higher than in normals despite the fact that the majority of the youngest hypertensives did not have higher peripheral resistance index at rest

In the two older groups, the resistance is elevated at rest in all but two (both < 50 years) During the highest work load 20 of the 24 in age group 40–49 years and *all* in the oldest group have higher values than anybody in the control group One of the two who has normal resistance

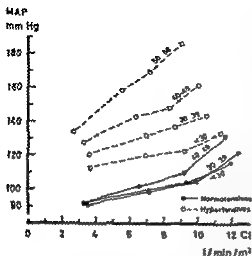


Fig 33 The pressure flow ratio at rest and during *exercise at 300 600 and 900 kpm/min* in various age groups

at rest also has normal resistance during work It is interesting to note that 3 of 4 hypertensives in the oldest group have a resistance index during the highest work load of the same magnitude as the controls at rest In other words the total diameter of the resistance vessels in a state of great vasodilation is the same as in controls in resting conditions! The TPRI increases significantly with aging The significance of the differences between the age groups is greater than for any of the other hemodynamic parameters

There are few observations on the TPRI *during work in the sitting position in hypertensives particularly in young subjects* with early *hypertension* Sannerstedt (134) reported originally that the TPRI during exercise in males below 30 years was not different from the control group in contrast to the findings reported from this laboratory in 1965 (110) However in his most recent work Sannerstedt (135) found that men in stage I had elevated resistance during work but not at rest His study however, only comprises 4 men under 30 years with essential hypertension and did not particularly aim at the early phase Amery *et al* (2) reported that the TPRI was elevated in hypertensive subjects < 35 years both at rest and during work If we study the pressure flow curves (fig 33) we see that the pressure rises most steeply in the oldest hypertensive group This is consistent with Sannerstedt's finding (135) The figure also demonstrates that the flow *at 900 kpm load falls decade by decade in* the hypertensives However, owing to the *very high resistance during work in the* oldest group the MAP is highest in spite *of the lowest flow*

g) Left ventricular work index (Table 35 and fig 34)

The LVWI increases almost linearly in all groups

Normotensives The increase in the LVWI from rest (table 17) to the 900 kpm load in the three age groups is 14 67 (356 %) 16 31 (374 %) and 16 73 kpm/min/m² (410 %) The relative increase in the work of the heart in transition from rest to the 900 kpm load is about half of the relative increase in the oxygen uptake There is a tendency for the LVWI to increase with aging but the difference between the groups is not significant

Hypertensives *In all age groups the* LVWI is higher than in the controls at *the lowest work level but with increasing load the difference from the controls be*comes less pronounced At the highest work load it is not significantly different from the controls and in the age group 30–39 years the LVWI is even a little lower The increase in the LVWI from rest to the 900 kpm/min load is 14 80 (260 %) 14 58 (247 %), 16 22 (291 %) and 17 69 kpm/min/m² (401 %) Thus the absolute increase in the LVWI is about the

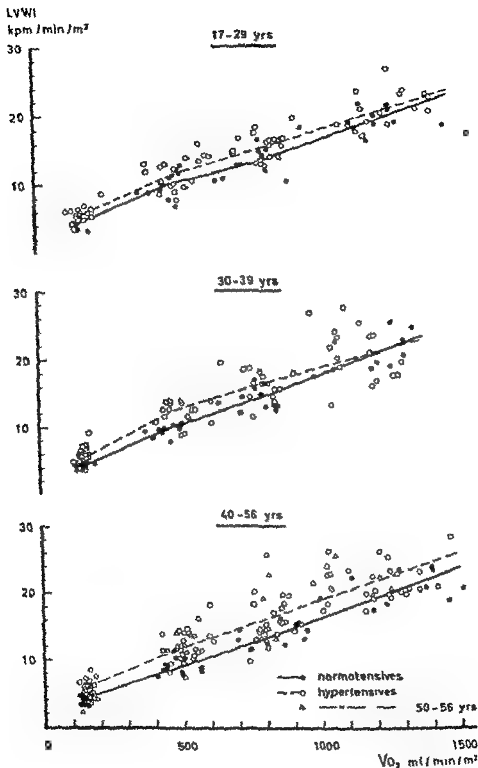


Fig 34 The left ventricular work index at rest and during work Legend as in fig 29

Table 35 *Left ventricular work index during work (kpm/min/m²)* (*Legend as in table 27*)

Age group	300 kpm/min Mean SD		600 kpm/min Mean SD		900 kpm/min Mean SD	
	NT	HT	NT	HT	NT	HT
1	9.8	11.3*	13.1	15.3**	18.8	20.5
	1.8	2.2	2.2	1.9	1.5	2.7
2	9.5	12.5***	14.1	16.2	20.7	20.5
	0.8	2.3	1.6	3.5	2.5	3.7
3	9.0	12.3***	13.7	16.8**	20.8	21.8
	1.2	2.2	1.4	3.6	2.2	2.7
4		12.0***		15.8		22.1
		2.1		3.5		2.3

Table 36 *Left ventricular stroke work index during work (gm/stroke/m²)* (*Legend as in table 27*)

Age group	300 kpm/min Mean SD		600 kpm/min Mean SD		900 kpm/min Mean SD	
	NT	HT	NT	HT	NT	HT
1	102.6	107.5	106.3	112.8	117.2	119.9
	16.4	17.7	13.7	14.8	10.2	17.6
2	99.9	114.9*	115.4	122.6	129.6	127.4
	10.3	19.1	12.9	23.4	11.1	25.7
3	99.5	117.8*	116.5	124.8	133.9	135.4
	15.4	21.9	13.2	24.5	11.7	24.7
4		125.9*		132.0		145.0
		28.4		40.2		16.8

same as in the controls but in the 3 youngest groups the relative increase is less There are no significant differences between the LVWI in the various age groups at any work level

h) Left ventricular stroke work index.
(Table 36 and fig 35)

In transition from rest to exercise the LVSWI rises in all groups studied and then curves off less steeply

Normotensives In transition from rest (table 18) to 900 kpm load the LVSWI increases 56.4 – 65.1 and 73.0 gm/stroke/m² or 93 – 101 – 120 % in the three age groups Table 36 shows that at the two highest loads the LVSWI tends to increase with aging but not significantly

Hypertensives In transition from rest to the 900 kpm load the LVSWI increases 48.0 – 54.3 – 62.2 and 85.7 gm/stroke/m² or 67 – 74 – 85 and 145 %. At the lowest work level the LVSWI is almost significantly higher than in the controls At the highest work level, the difference from the controls is slight At all work levels the LVSWI tends to increase with aging, but not significantly

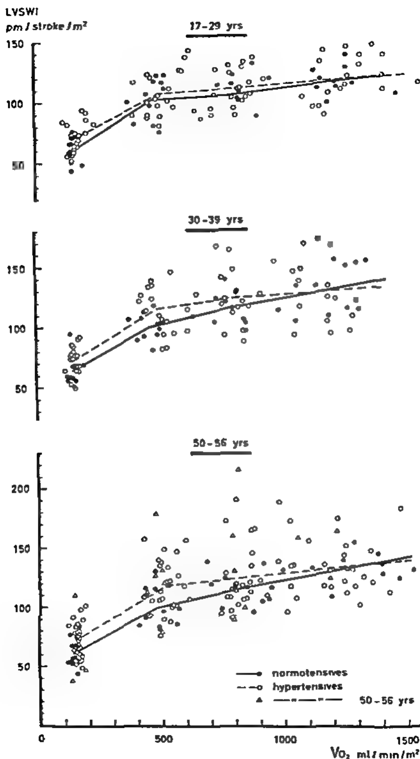


Fig 35 The left ventricular stroke work index at rest and during work. Legend as in fig 29

Table 37 *Arteriovenous difference during work (ml/l) (Legend as in table 27)*

Age group	300 kpm/min Mean SD NT	HT	600 kpm/min Mean SD NT	HT	900 kpm/min Mean SD NT	HT
1	65 8	73 7	85 3	89 6	100 6	112 6*
	9 3	13 2	13 5	9 2	11 8	13 9
2	64 4	73 4 *	78 6	91 1**	95 4	112 3**
	8 8	9 7	11 4	9 9	10 9	17 7
3	74 3	81 7*	90 2	103 1**	112 0	122 0
	10 0	9 1	9 2	12 2	15.2	14 9
4		89 2*		119 2***		137 0*
		13 9		21 0		27 3
	2–3*	2–3*		1–3** 2–3** 3–4*	2–3*	

1) **Arteriovenous difference** (Table 37 and fig 36)

The arteriovenous difference increases with increasing load most pronounced in transition from rest to light exercise

Normotensives In transition from rest (table 19) to the 900 kpm load the arteriovenous difference increases 55 6 – 53 2 and 68 8 ml/l or 124 – 126 and 159% in the three age groups Table 37 shows that there is a tendency to increased arteriovenous difference during work with aging after 30 years The difference between the two oldest groups is almost significant at the 300 and 900 kpm loads

Hypertensives In transition from rest to the 900 kpm load the arteriovenous difference increases 68 0 – 69 9 – 72 3 and 74 8 ml/l or 152 – 165 – 145 and 120% in the four age groups

During work the arteriovenous difference tends to be higher in the hypertensives In the youngest group the difference from the controls is almost significant at the 900 kpm load In the three oldest groups the difference from the controls is greater and significant (or almost significant) at all work levels except the highest level in the age group 40–49 years

As in the normotensives, the arteriovenous difference tends to increase with aging The differences between the groups is most marked at the 600 kpm load

2 Comments on the results from the observations during exercise

The changes in the cardiovascular system in transition from rest to so-called steady state exercise in normal man are dependent upon many factors of which sex (14 135, 167) age (80, 99) physical training (60 73) – type of work (152) and body position (17 160 161) are important It seems quite clear from the present results compared with previous investigations that when the effect of a disorder (such as arterial hypertension) is going to be studied, a control group of the same age sex habitual and actual physical activity and submitted to exactly the same experimental procedure is mandatory

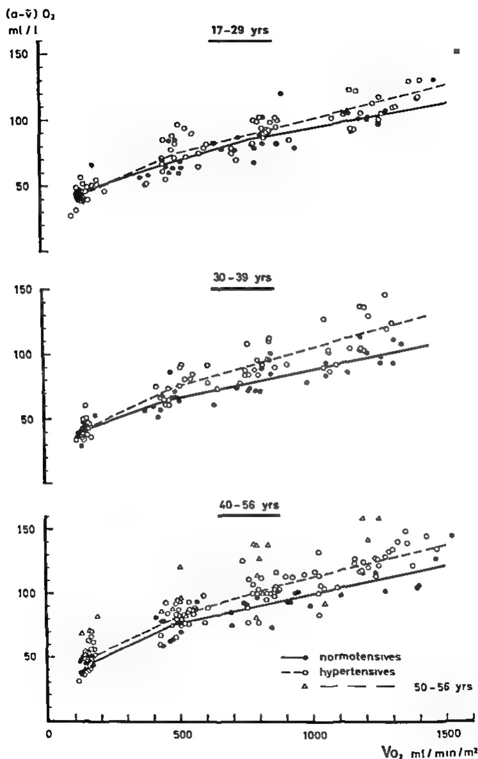


Fig 36 The arteriovenous difference at rest and during work Legend as in fig 29

In the normotensives (Norwegian men aged 17–49 years) who took part in this study, the heart increased its output by a rise in frequency and stroke volume, both in young and old subjects The total vasodilation during work, *however, was signif icantly* reduced by aging and the work of the heart expressed as LVWI and LVSWI, increased with aging (although not significantly) The normotensive s heart was able to maintain adequate pump function with aging in spite of the resist ance during work increasing with aging, and consequently also in spite of the rise in the brachial artery pressure These general results which are the essential findings in the normotensives, compare well with recent results from other labo ratories

The absolute values of heart rate, cardiac output, MAP and TPR in relation to work load or oxygen uptake differ from many studies The cardiac output and SV was in the upper range of reported values The differences can probably be explained by different selection and physical activity in the controls and slightly different procedures According to the model experi ments (page 20) overestimation of the CO seems unlikely

The first main question in this part of the study was whether the total peri pheral resistance during work in the young-est hypertensives would remain no differ ent from that in the controls The results demonstrate that, already in transition *from rest to light work, the total peri* pheral resistance is *higher* than in the con trols in *all* age groups including the youngest and it remains higher during heavier work The results also show that the differences in the resistance between the hypertensive age groups increase dur ing work

The next main question concerns the heart pump function The results show that the blood flow during work is *lower* in hypertensives than in controls in all age groups but the difference is slight and not significant in the youngest group

The results also demonstrate that the CI during work decreases significantly with aging in the hypertensives in con trast to the controls

Since physical exercise does not lead to an augmented pump response in the young hypertensives, the regulation mechanisms seem to function normally in response to this stimulus At least they do not allow an abnormally high cardiac output in relation to work load or oxygen need The subnormal CO in the hypertensives is due to a significantly reduced SV — consistent with findings by other workers (135, 158)

The reduced SV is partly compensated by elevated heart rate consistent with other workers (135)

A reduced cardiac output in relation to work load and oxygen need is characteristic in heart failure (83 113, 140) None of the subjects in this study had symptoms or clinical signs of heart failure The simplest explanation of the subnormal CO in relation to oxygen need is that, if a normal CO should be pumped against the elevated peripheral resistance the work of the heart would be too great If the organism tries to protect the heart from too much overstrain in the presence of high systemic resistance it can be done by reduction of the output and if the arteriovenous dif *ference increases to the same extent, the* O_2 supply to the tissues will not suffer

The left ventricular stroke work in the hypertensives during exercise is only slightly different from in the controls — during moderate and severe work none of the differences are significant Owing to a compensatory increase in frequency the left ventricular work per minute is

higher than in the controls during light and moderate work but, *during severe work*, the difference from the controls is not significant

Thus it seems that the reduced pump function of the heart expressed as pumped blood flow in relation to oxygen need — could be due to a safety mechanism, trying to protect the heart from overstrain

A study in humans has shown that the reduction of an elevated peripheral resistance by phentolamine causes a greater CO during a work load than when no drug is given (50) This has a great therapeutical significance If a drug could be found which really reduces the peripheral resistance in hypertension — without interfering with venous return or heart function — it seems reasonable that the hemodynamics in hypertension during work could be normalized — at least as long as no permanent damage has occurred in the heart pump

The "safety mechanism" to which the hypertensives resort in order to meet the oxygen demands from the tissue, is to increase the extraction of O_2 from the blood

It is shown that the arteriovenous difference is higher in the hypertensives than in the controls, most marked at the highest work level

It is clear that since the cardiac output was lower, and the arteriovenous difference higher, the "oxygen reserve" in the blood — the amount of O_2 not used and transported back to the lungs — is reduced in the hypertensives In this respect they resemble patients with heart diseases (48)

In conclusion, it might be stated that the hemodynamic response to exercise in men with essential hypertension is more complex than in normotensives The results demonstrate an increased total peripheral resistance in *all* age groups, including *the youngest The pump function of the heart is subnormal, probably because it is restricted by the elevated peripheral resistance during work, and this is seen already in the hypertensive men in their twenties*

C Studies after dextran infusion

1 Results

a) Before infusion Table 38 summarizes the findings before infusion As expected, the CI is highest in the hypertensives < 40 years and lowest in the hypertensives

Table 38 *Hemodynamic parameters before Macrodex infusion (rest, supine)*

	Normotensives			Hypertensives					
	< 40 years (n=10)			< 40 years (n=9)			> 40 years (n=9)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
CVP mmHg	1 1–4 8	3 3	1 0	0 7–5 9	3 4	1 5	0 1–5 9	2 4	1 7
HR beats/min	49–80	61 1	7 5	50–78	65 0	9 7	51–90	68 2	11 9
CI l/min/m²	2 98–4 22	3 39	0 43	3 18–4 83	3 68	0 50	2 44–4 21	3 30	0 48
SI ml/stroke/m²	46 9–69 1	55 7	6 3	43 4–69 0	57 2	7 8	41 6–64 5	49 1	6 9
SAP mmHg	100–122	112 3	5 6	113–159	135 7	11 8	136–182	159 4	14 8
DAP mmHg	60–76	66 1	5 3	67–98	84 7	9 4	79–114	92 4	12 0
MAP mmHg	77–93	84 1	5 1	96–123	103 9	10 0	101–135	118 4	10 9
TPRI dynseccm⁻⁵m²	1636–2233	2011	225	1755–3094	2301	396	2052–4196	2950	629

Table 39 *The changes in CVP, CI HR, SI MAP and TPRI after the infusion (+) of 500 and 1000 ml dextran and then removal (—) of 500 ml blood (Reference values = status before infusion Figures in italics mark the change in per cent of the reference value)*

		+ 500 ml			+ 1000 ml			— 500 ml		
		NT	HT <40 years	HT >40 years	NT	HT <40 years	HT >40 years	NT	HT <40 years	HT >40 years
n		10	9	9	10	9	9	10	9	8
Δ CVP (mm Hg)	Mean	3.64	3.71	3.08	6.29	6.99	5.43	2.03	1.72	1.62
	SD	1.2	1.2	0.8	1.7	0.9	1.5	1.4	1.8	1.6
		111	*109*	*129*	*192*	*206*	*227*	*62*	*51*	*67*
Δ CI (l/min/m^2)	Mean	0.23	0.62	0.31	0.54	0.94	0.64	0.74	0.69	0.51
	SD	0.24	0.57	0.19	0.51	0.76	0.64	0.48	0.46	0.37
		6.9	*16.8*	*9.5*	*16.0*	*25.6*	*19.3*	*21.8*	*18.8*	*15.3*
Δ HR (beats/min)	Mean	−1.1	2.3	−0.1	0.5	4.2	3.1	4.7	2.4	3.1
	SD	2.8	3.1	1.8	4.0	6.9	6.1	5.6	5.4	6.3
		−1.8	*3.5*	*−0.1*	*0.8*	*6.5*	*4.5*	*7.6*	*9.5*	*4.5*
Δ SI (ml/stroke/m^2)	Mean	5.1	6.5	4.9	8.4	10.3	7.0	7.1	9.2	5.0
	SD	5.4	8.8	3.1	7.3	8.2	5.8	6.1	7.2	2.0
		9.2	*11.4*	*10.0*	*15.1*	*18.0*	*14.2*	*[illegible]*	*16.0*	*10.1*
Δ MAP (mm Hg)	Mean	3.0	4.5	3.7	6.6	8.4	8.3	5.3	4.4	4.1
	SD	1.0	4.9	9.6	4.6	5.3	8.2	4.6	5.0	8.7
		3.6	*4.3*	*3.1*	*7.8*	*8.1*	*7.0*	*6.3*	*4.2*	*3.4*
Δ TPRI (dyn [illegible] cm^{-5}m^2)	Mean	−53	−204	−192	−119	−250	−201	−221	−206	−353
	SD	193	323	236	324	288	426	237	212	283
		−2.6	*−8.9*	*−6.5*	*−5.9*	*−10.9*	*9.5*	*−11.2*	*−12.4*	*−12.0*

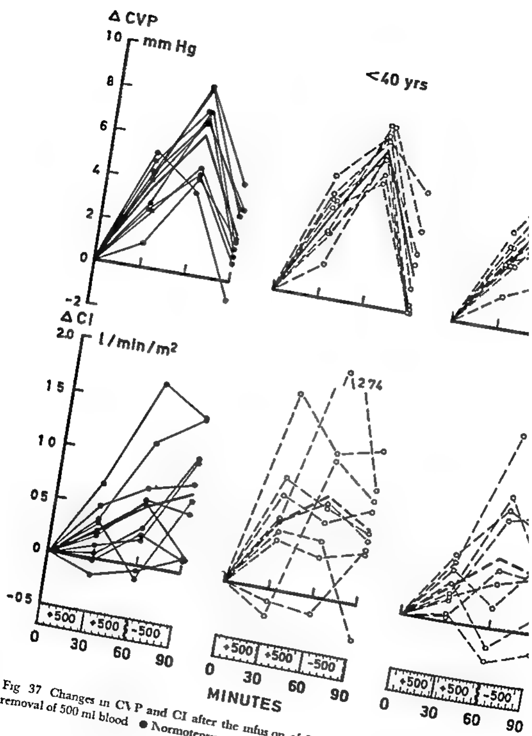


Fig 37 Changes in CVP and CI after the infusion of 500 + 500 ml dextran and subsequent removal of 500 ml blood ● Normotensives ○ Hypertensives The thick lines show mean values

> 40 years The SI is lowest in the older hypertensive group and similar to the control value in hypertensives < 40 years The TPRI is highest in the hypertensives > 40 years The CVP is similar in all groups The body weight (table 6) is higher (although not significantly) in the hypertensive groups than in the controls and therefore the infused volume of Macrodex per kg bodyweight is less in the hypertensives -

b) After infusion and bleeding

b1) *CVP* The changes in the CVP after infusion and bleeding, are shown in table 39 and figs 37 and 41 The responses are quite consistent and of similar magnitude in all the groups After the first infusion, the CVP rises about 3 - 5 mm Hg and after the second, it rises to about 10 mm Hg After bleeding, the CVP drops in all cases usually to values a little lower than those after the first infusion As seen from fig 41, the relative changes in the mean values are almost identical in the three groups and the differences between the two hypertensive groups and the normotensives are not statistically significant

b2) *Cardiac output* The changes in CI are shown in table 39 and figs 37 and 41 The changes are less uniform than those in the CVP

In the normotensive group, with two exceptions the CI rises after the first infusion After the second, it rises in all but one The spread of the individual values is considerable, particularly after the last infusion The mean values of the absolute and relative changes after the first and second infusions are + 0 23 l/min/m² (+ 6 9%) and + 0 54 l/min/m² (+ 16 0%) After the bleeding the response varies in 4 there is a fall in 6 a rise As seen from fig 37 there is a rise in the mean values after the bleeding

In the hypertensive groups, particularly the younger, the responses vary considerably In the younger group, the changes after the first infusion range from — 0 11 to + 1 8 l/min/m², mean + 0 62 (+ 16 8%) and after the second, they range from — 0 16 to + 2 74 l/min/m², mean + 0 94 (+ 25 6%) Thus the mean increase is somewhat higher than in the controls but owing to the great scatter in the individual values none of the changes are significantly different from the controls (The greater difference was after the first infusion but the P value was only 0 09)

With regard to individual responses it is seen from fig 37 that after the first infusion 6 of the 9 young hypertensives against only one of the 10 normotensives have an increase above 0 5 l/min/m²

After bleeding the findings are inconsistent with a fall in the mean value as seen from fig 37

In the older hypertensive group there is a consistent increase in the CI after the first infusion range - 0 07 to + 0 62 l/min/m² mean + 0 31 (+ 9 5%) Only in two does the increase exceed 0 5 l/min/m² After the next infusion the range is — 0 30 to + 1 78 l/min/m , mean 0 64 (+ 19 3%)

Thus the increase in this group is somewhat lower than in the younger hypertensive group and a little higher than in the controls The difference from the results in the control group is not significant (as expected since the deviation from the controls is even less than in the younger group)

After bleeding the responses are inconsistent

b3) *Relationship between the CVP and the CI* There is no relationship between the changes in the CVP and the CI in any of the groups as seen from fig 38

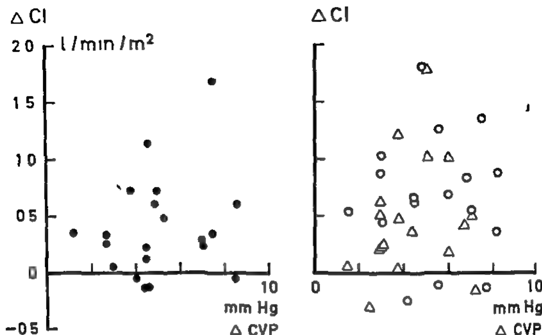


Fig 38 The relationship between changes in CVP and CI ● Normotensives
○ Hypertensives < 40 years △ Hypertensives > 40 years

b4) *Heart rate* The changes are shown in table 39 and figs 39 and 41 In the normotensive group the changes after the infusion are inconstant, and the mean value is almost unchanged After the bleeding there is usually a rise in the heart rate and an increase in the mean value

In the young hypertensive group the responses also varied, from — 2 to + 7, mean +2 3 (+ 3 5%) after the first infusion, and from — 2 to + 22, mean +4 2 (+ 6 5%) after the next Thus there is a slight increase in the mean value, in contrast to the controls The difference is not significant After the bleeding, the findings are inconstant

In the older hypertensive group, the responses do not show any particularly consistent pattern

b5) *Stroke volume* The changes in the stroke index are shown in table 39 and figs 39 and 41 The individual responses vary considerably but are more consistent than the changes in the heart rate

In the normotensives, there is a rise in all but two after the first infusion, range — 2 1 to + 18 6 ml/stroke/m², mean + 5 1 (+ 9 2%) After the next infusion, there is a further increase in all but one, range — 2 1 to + 25 0 ml/stroke/m², mean + 8 4 (+ 15 1%) After the bleeding, the changes are inconstant

In the young hypertensive group, the SI rises in all but one after the first infusion, then the response varies After the first infusion the changes range from — 11 3 to + 21 4 ml/stroke/m², mean + 6 5 (+ 11 4%) after the next the range is — 6 4 to + 24 3 ml/stroke/m², mean + 10 3 (+ 18 0%) Thus the increase is only slightly higher than in the controls After the bleeding, the stroke volume usually fell

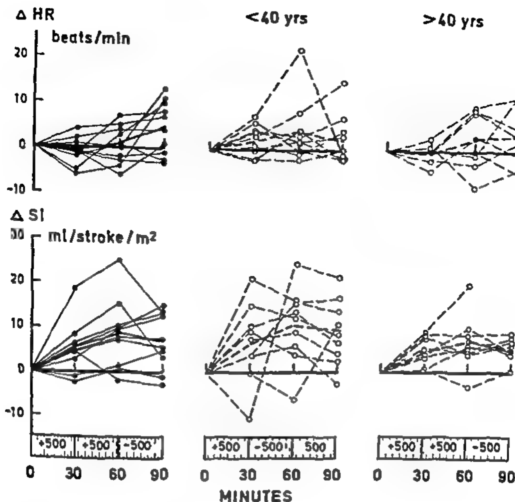


Fig 39 Changes in HR and SI after infusion of 500 + 500 ml dextran and subsequent removal of 500 ml blood. Legend as in fig 37

In the older hypertensive group the response varies less After the first infusion the SI increased from + 0 7 to + 9 4 ml/stroke/m² mean + 4 9 (+ 10 0%) After the next the changes range from + 3 4 to + 10 0 ml/stroke/m² mean + 7 0 (+ 14 2%) The increase is nearly identical with that in the control group After the bleeding there are small changes

The changes in the two hypertensive groups are not significantly different from those in the controls

b6) *Mean arterial pressure* The changes are shown in table 39 figs 40 and 41

In the normotensive and younger hypertensive group the changes are rather consistent but in the older hypertensive group they vary considerably After one or two infusions the mean blood pressure usually rises 2 15 mm Hg in all groups The rela

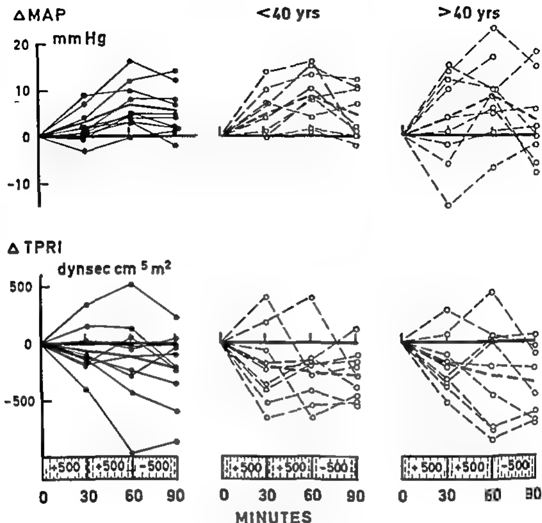


Fig 40 Changes in MAP and TPRI after the infusion of 500 + 500 ml dextran and subsequent removal of 500 ml blood Legend as in fig 37

tative changes in the blood pressure are almost identical, as seen from fig 41, and there are no significant differences between the responses in the three groups

b7) *Total peripheral resistance* As seen from fig 40 the changes in the TPRI vary considerably but in the hypertensive groups, there is usually a drop after the infusions as well as after the bleeding After the infusions the mean values show a somewhat *greater* fall in the hypertensives (fig 41) After the bleeding, the reduction of the resistance is similar in all There are no significant differences between the responses in the three groups

If we compare the hemodynamic pattern before and after dextran infusion in the hypertensives under 40 years with controls, we find that before infusion, the mean arterial blood pressure in the hyper

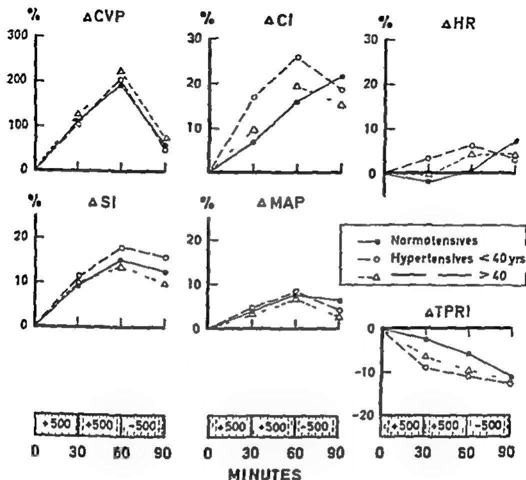


Fig 41 Survey of the relative changes in CVP CI HR SI MAP and TPRI after the infusion of 500 + 500 ml dextran and subsequent removal of 500 ml blood

tensives is 23 5 % higher, the flow 8 5 % higher and the peripheral resistance 14 4 % higher After the first dextran infusion the mean arterial pressure is 24 5 % higher almost no increase in the difference from the situation before the infusion) the flow 18 8 % higher and the resistance only 7 1 % higher Thus there has been a fall in the peripheral resistance great enough to avoid an abnormal pressure rise from the greater increase in flow After the next infusion the differences are mainly the same as after the first infusion In other words, the artificially increased blood flow does not demonstrate any reduced conductance in the arterial bed in the younger hyper tensives – in contrast to the (far greater) increase in the flow induced by exercise

2 Comments

The plasma expanding effect of dextran was not measured in this study but, in normal men, Hammarsten *et al* (78) found that one hour after the infusion of 1000 ml 6% dextran in saline, given over a period of 50 minutes, the plasma volume had increased 775 ml The total volume and rate of infusion was similar in the present study, so it is reasonable to assume that the plasma volume in the controls had increased about $^3/_4$ l at the time of the second measurement

The infusion caused a consistent increase in the *CVP* of about 5–8 mm Hg in the normals in agreement with several investigators (62, 137) The increase in the CVP in the hypertensives was no greater than in the controls, in contrast to the findings by Ulrych *et al* (157) The number of patients in whom they measured the CVP during dextran infusion was, however, limited to 4 Thus, the present study does not support the assumption of a reduced venous capacity in the hypertensives It could of course be argued that even if the CVP did not rise more in the hypertensives than in the normals, there might be a decreased expanding capacity if the infused fluid were excreted quicker in the hypertensives, so that the CVP was higher in the presence of a smaller intravascular volume This cannot be excluded, as neither plasma volume nor diuresis was measured in this study

The observations on the *cardiac output* in the normals agree well with observations by Schnabel *et al* (137) They found that during dextran infusion, which caused a rise in right atrial filling pressure of about 2–12 mm Hg, the CO and SV increased about 30% and 20% respectively These figures were found after the infusion of a somewhat larger dextran volume than was used in the present experiment They found no significant relation between the magnitude of the increase in right heart filling pressure and the percentage increase in CO and SV The present findings are in accordance with this

The normal response to hypervolemia – a consistent increase in CVP and right atrial filling pressure and a less consistent rise in CO and SV – not closely related to the venous pressure changes – suggests that factors other than filling pressure are important for the control of the cardiac output in normal, non medicated man (36 62, 137)

The first main question in this part of the study is whether the *CO* would increase more in hypertensives than in normotensives, and whether the increase would be most pronounced in the youngest as suggested by Ulrych *et al* (157) The present results point in the same direction as their findings, although the individual responses varied considerably and the difference from the controls is not statistically significant The deviation from the control group is considerably less than in Ulrych *et al* s experiment However, they reported the maximal changes seen during the infusions and gave about 20% more dextran They also report considerably higher resting CI in the controls – 4 57 l/min/m² – against 3 39 in this study This could be due to a greater experimental stress in their study since bladder catheterization was also performed

The insignificantly greater cardiac response in the hypertensives cannot be explained by an abnormal rise in the CVP – as suggested by Ulrych *et al* (157) Since it has been shown that the CO response to hypervolemia in normal man can be greatly increased if the autonomous nervous system is blocked by drugs (62), the observations in the hypertensives could

be explained by a disturbed or less efficient autonomous regulation of the heart

Recently a report by Welner and Groen (163) showed that the exaggerated diuretic and natriuretic response to salt loading in hypertensives could be normalized by a simple deconditioning procedure This suggests that the increased diuretic and natriuretic response could be explained simply on a *psychogenic* basis Many workers have shown a greater cardiovascular response to many types of physical (10, 87, 141) and psychogenic stimuli (27, 28 43) in subjects with essential hypertension It is therefore not unlikely that the greater increase in the CO (and in the diuresis) following plasma expansion could simply be due to a greater general reactivity in the hypertensives to many types of stimuli or stress The present results could support this view as the subject knew what was going to happen and no attempt was made to hide the Macrodex bottles

The second main question in this study concerns the peripheral vessels The hypertensive s greater increase in cardiac output after fluid loading was not associated with any greater increase in the arterial blood pressure, and restricted vasodilation was not demonstrated in the hypertensives

The effect of the *bleeding procedure* is more difficult to interpret since reduction in the CVP usually reduces the CO (114) while reduction in the erythrocyte volume leads to an increase (114), These oppo ing mechanisms were possibly responsible for the inconsistent results

Venesection causes a consistent drop in the right atrial pressure but the cardiac response is inconsistent (162) In both the hypertensives and the controls the CVP fell and so did the mean value of the CO in the two hypertensive groups but it is peculiar that the mean value of the CO rose in the normotensives If we suppose that the hypertensive subjects were more sensitive to CVP pressure changes, and the normotensives more sensitive to changes in the red cell volume, the reaction patterns could be explained

It must be emphasized that the changes observed in this experiment are usually small, and that none of the differences between the controls and the two hypertensive groups are significant Furthermore the differences between the hypertensive groups < 40 and > 40 years are even less

It is therefore not possible to draw unanimous conclusions from this experiment only to demonstrate certain tendencies

C Studies after food intake

1 Results

Since the number of subjects is so small statistical calculations are omitted

a) Before meal

The results are shown in table 40 The HR tends to be higher in the hypertensives the CI is nearly the same in the hypertensives and the controls and consequently the SI is a little lower in the hypertensives The total peripheral resistance is as expected higher in the hypertensives than in the controls (all hypertensives > 35 years)

b) After meal

b1) *Central venous pressure* showed no changes

b2) *Cardiac index* Fig 42 shows that the CI increases in all subjects In the normotensive group the increase ranges from 0 46 to 0 69, mean 0 59 l/min/m² In the hypertensives the individual changes

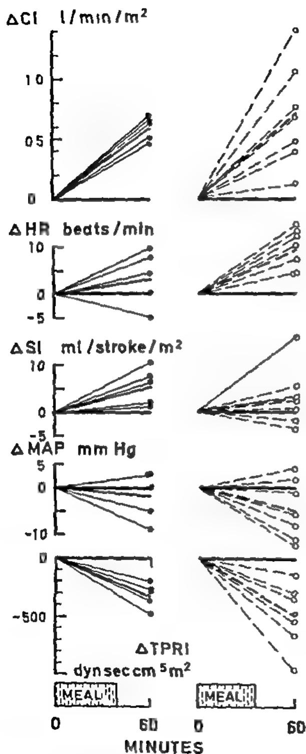


Fig 42 Survey of the changes in CI, HR, SI, MAP and TPRI after a heavy meal ● Normotensives ○ Hypertensives

Table 40 *Hemodynamic parameters before heavy meal (rest supine) (NT — normotensives HT — hypertensives)*

		NT (n = 5)		HT (n = 7)	
		Range	Mean	Range	Mean
HR	beats/min	60–85	68 0	50–100	72 2
CI	l/min/m²	3 33–3 62	3 49	2 11–4 31	3 5?
SI	ml/stroke/m²	42 5–59 8	52 1	4? 2–57 7	48 8
SAP	mmHg	103–121	115 8	131–205	161 3
DAP	mmHg	56–76	69 0	79–108	93.2
MAP	mmHg	72–90	86 6	101–139	118 8
TPRI	dyn sec cm⁻⁵m²	1604 2162	1986	2000–5270	2887
LVWI	kpm/min/m²	3.51–4 77	4 10	3 98–7 79	5 63

show a greater variation from 0 11 to 1 40 mean 0 69 l/min/m² Thus the absolute changes are almost the same in the hypertensives and the controls and since the preprandial values are nearly the same the relative increases are also almost the same 16 9% in the normotensives and 19 7% in the hypertensives (table 41)

b3) *Heart rate* The HR rises in 3 of the normotensives and in all hypertensives (Fig 42) and the mean increase is 5% in the controls against 12 6% in the hypertensives

b4) *Stroke index* The SI rises in all normotensives and in all but 2 hypertensives (fig 42) The mean increase is slightly higher in the normotensives (table 41)

b5) *Mean arterial blood pressure* As seen in fig 42 the MAP shows inconsistent changes in both groups but it usually falls The fall is slightly more pronounced in the hypertensives than in the controls 3 9% against 1 8% (table 41)

b6) *Total peripheral resistance* Fig 42 shows that the TPRI falls in all subjects In the normotensive group the fall ranges from 202 to 483 mean 320 dyn sec cm⁻⁵m² The relative fall in the mean values is almost identical in the two groups 16 1% in the controls and 16 9% in the hypertensives Before the meal the mean value in the hypertensives was 45 3% higher than in the controls after the meal 44% higher Thus the absolute fall in the TPRI is greatest in the hypertensives and the resistance after the meal is relatively no higher than before

Table 41 *The changes in CI HR SI MAP TPRI and LVWI after a heavy meal (Figures in italics mark the change in per cent of the preprandial value)*

		NT (n=5)	HT (n=7)
Δ CI(l/min/m²)	Mean	0 59	0 69
		16.9	*19 7*
Δ HR(beats min)	Mean	3 4	9 1
		5 0	*12 6*
Δ SI(ml stroke/m²)	Mean	5 5	3 0
		10 6	*6.2*
Δ MAP(mm Hg)	Mean	— 1 6	—4 6
		— 1.8	*— 3.9*
Δ TPRI(dyn.sec.cm⁻⁵m²)	Mean	320	—488
		—16 1	*16.9*
Δ LVWI(kpm/min/m²)	Mean	0.58	0 77
		14 1	*13 7*

b7) *Left ventricular work index* The LVWI rose in all subjects The mean rise is almost identical in the two groups, 14 1% in the controls and 13 7% in the hyper tensives (table 41)

2 Comments

This study must be interpreted as a small pilot experiment and the small number of subjects and heterogenous hypertensive group (4 in stage I and 3 in stage II) implies that only very limited conclusions may be drawn The main purpose of this experiment was to see whether the total peripheral resistance in the hypertensives would differ more from the controls after a meal than before, suggestive of a particularly high vascular resistance in the hepatosplanchnic area It has been stated that the vascular resistance is increased very early in the reno hepato splanchnic region in essential hypertension (65)

The hypertensive group contains sub jects in stage I, over 35 years, and subjects in stage II, and regional vascular changes should therefore be expected in them All of them had higher TPRI than the highest value in the control group, but after arbitrary borderlines (page 40) only 4 had "high" resistance, the others "medium"

The intake of the heavy meal causes a similar increase in the cardiac output of about 17–20% in both hypertensives and controls This increase is expected owing to increase in the metabolism Absorption of fluid and increase in the plasma volume may also contribute to the increase

The increase in the blood flow did *not* result in a rise in the blood pressure, on the contrary – the blood pressure tended to drop The total peripheral resistance dropped more than was necessary to maintain the same blood pressure as before the meal The relative changes in the TPRI are similar in hypertensives and controls

Why the vascular resistance drops more than necessary to maintain the same blood pressure as before the meal, cannot be answered by this experiment, as no regi onal measurements of the flow and resist ance were made However, if we assume that most of the increase in flow passes through the reno hepato splanchnic re gion, the results do *not* point to a particu larly high resistance in this region But of course it cannot be excluded that compensatory vasodilation could take place in other vascular regions and mask the changes in the reno hepato splanchnic area It is possible that the method is too crude for the purpose of demonstrating the effect of changes in the regional circulation when the local flow is relatively small in comparison with the cardiac out put Nevertheless the results demonstrate that subjects with essential hypertension in stage I and II can tolerate a heavy dinner without any rise in the blood pressure On the contrary, the meal reduces the peripheral resistance and the pressure usually drops a little The work of the heart increases relatively no more than in controls

As the results did not show any differences between these hypertensives and controls, the study was not extended to examine the effect in younger hypertensives separately

V General discussion and conclusions

In all clinical research work, selection of the patients and the criteria used for their classification are highly important for the conclusions which can be drawn This is particularly true when dealing with a disorder like essential hypertension which is usually of long duration through various stages and is not diagnosed by any specific tests but simply by the principle of exclusion

This study has had the advantage that most patients were selected from a population study or from files providing medical information on several thousand men in active work in various occupations Even if the subjects were not strictly randomised, they should represent an unselected group of men with at least 3 successive casual BP readings above 140/90 mm Hg – the upper limit for normotension proposed by WHO (7)

The diagnosis of essential hypertension in this study was established after thorough examination

However in view of the possible complications of renal arteriography it was not considered justifiable to perform this on all the hypertensives even though it was desirable from a scientific point of view Thus it may be argued that renal artery stenosis could have been present in some of the patients All the patients had normal serum electrolytes, and consequently not the usual Conn s syndrome It is a theoretical possibility that a few of the subjects could have a normokalemic aldosteronoma as the cause of their hypertension (33) but this is probably very rare in unselected hypertensive subjects 107) Even though the aldosterone secretion rate was rather high in some of the patients in whom it was measured (112) nobody had values suggestive of aldosteronism There is also a slight theoretical possibility that one or more of the patients could have pheochromocytoma as only one 24 hour urine sample was examined for catecholamines However the probability of including subjects with secondary hypertension should be very small and it thus seems justifiable to assume that the patients in the study comprise a representative group of subjects with essential hypertension With few exceptions all were untreated and ambulatory and all were without serious complication or other diseases To facilitate international comparison, they were classified as suggested by WHO (7)

The main interest of this study is the hemodynamic status in the starting phase of essential hypertension The problem was therefore to find subjects in this phase The ideal way would have been to examine a group of subjects with a long series of reliable blood pressure readings which had been normotensive for years but had recently started to rise, without

any demonstrable cause Such subjects are, however, difficult to find In this study another approach was chosen – to examine subjects of various ages, on the assumption that the youngest group will mainly contain subjects in the starting phase and the older group mainly subjects with longer duration of the disorder The problem was simplified a bit by the fact that essential hypertension rarely begins after the age of 50, usually in the twenties to forties (13, 149)

The actual duration of hypertension was impossible to assess in a large proportion of the subjects in this study However, table 2 which is based on available data *for subjects in stage I shows that, in the age* group 17–30 years, only 3 of 21 had a known duration of 10 years or more, whilst in the age group 40–49 years this was true in more than half of the subjects

In this group, we have 8 subjects with documented hypertension for 15 years or more, and who were already hypertensive in their twenties It is therefore likely that, by this approach we have succeeded in finding a group of hypertensives without complications (stage I) in which the duration is mainly short for the youngest group and more long standing for the older groups

The most important question in this study is whether the high blood pressure in young subjects with essential hypertension is maintained by a high cardiac output *in the presence of a normal* total peripheral resistance in contrast to the situation in the subjects in later stages of hypertension, where the resistance is expected to be high and the flow normal or reduced If this is true, and young subjects with essential hypertension change their hemodynamics towards the pattern seen in older patients, then it is likely that disturbances in the heart pump is of major importance in the pathogenesis of essential hypertension The increased resistance thought to be characteristic in late essential hypertension could then be secondary

What pattern emerges from the present study?

The findings *at rest* demonstrate that in hypertensive subjects under 30 years, the TPRI was *not* significantly different from in the controls of the same age, although the mean value was somewhat higher, most of the hypertensives had TPRI within the same range as the controls The blood flow expressed as CI was almost significantly higher than in the controls

The hemodynamic pattern in hypertensives under 30 years was different from the pattern in hypertensives 40–49 years, where the flow was no different from that in controls of corresponding age and the resistance was highly significantly higher than in the controls

In the age group 30–39 years, a more heterogeneous picture was found, some with the juvenile pattern, some with the pattern seen in the age group 40–49 years and some with both high CI and high TPRI – a possible transitory form between the two groups In the group > 50 years, the flow was lower than in the controls aged 40–49 years and lower than in hypertensives 40–49 years old The resistance was high This variation in the hemodynamic pattern at rest in hyper*tensive subjects at various ages, was not* seen in the normotensives

These observations point to a change in the hemodynamic pattern as the years go by, from a high flow-normal resistance in the early phase to a low flow high resistance in later and more advanced phases of the disorder If such a change is going to take place, showing that high flow normal resistance really is the char

acteristic disturbance in the early phase of essential hypertension, can of course only be proved or disproved by prospective studies It could be argued that the young men with hyperkinetic circulation may have had a different disorder from true essential hypertension, with normo or hypokinetic circulation and the expected high peripheral resistance, commonly observed in the men above 40 years It may also be argued that, in view of observations by others (21, 52, 98), it is unlikely that all the youngest will still be hypertensive in the future This does not rule out the possibility that they are at present in the early phase of essential hypertension because, in many chronic diseases, it is not unusual to find subjects who simply pass through the initial stage and then the pathological process stops The important question is whether the *majority of those who remain hypertensive* will change their pattern to a normo– or hypokinetic flow and a high peripheral resistance

If we turn to the 8 hypertensives aged 40–49 years who had been hypertensive since their twenties, we find the following hemodynamic pattern in them today high flow medium resistance 1, high flow high resistance 2, medium flow high resistance 3 low flow high resistance 2 This is nearly the same pattern as in the total group aged 40–49 years Thus nearly 90% of these subjects, hypertensive since their twenties, *now* had high resistance If essential hypertension was the same 15 years ago as today, which we must assume, then it is quite probable that most of these 8 have increased their resistance, since high resistance in 88% of a sample of men with essential hypertension in their *twenties* seems most unlikely in light of the findings in this age group today This of course does *not prove* the assumed change in the hemodynamic pattern in essential hypertension but seems to be a strong indication in favour of it

Many other studies have demonstrated a high CI and a normal TPRI in subjects with early hypertension (15 52, 53, 135) Since few or no studies have used unselected, ambulatory patients, it is not surprising that some workers have found a normal CI and high TPRI in such subjects (2)

It must therefore be concluded that many studies including the present one, have demonstrated that the *characteristic hemodynamic disturbance in early essential hypertension in men at rest, is a high cardiac output in the presence of a normal total peripheral resistance*

The high cardiac output is maintained at least in the sitting position by a high heart rate and a normal stroke index Thus the observations at rest indicate that the heart pump is responsible for the high blood pressure in young men with essential hypertension the pump works too fast

The observations from the *dextran infusion* experiment support this view and also point to a possible disturbed regulation of the heart pump in early essential hypertension The findings could fit with the assumption of a disturbed autonomous regulation of the heart (62)

They could also support the view that these disturbances were most pronounced in early hypertension but were still present to a minor degree in patients over 40 years The observations during *muscular exercise* and *after a heavy meal* however did not demonstrate any overactive heart function in those situations where the oxygen need is probably the most important factor determining the CO The study does not explain *why* the heart pump should be overactive in young hypertensive subjects at rest The CO was related to the meta

bolism, in contrast to the situation in the hyperkinetic syndrome described by Gorlin (66) where there was true luxury perfusion This hemodynamic pattern resembles the pattern seen during acute fear and anxiety (30, 39, 82, 86, 151, 165) The findings could be consistent with the Russian concept that essential hypertension is a psychosomatic disease (116, 144) and that, in the early phase, the nervous tension mainly affects the heart

As the years go by, the resistance in the vessels increases due to autoregulation (71) A psychiatric examination of the hypertensives and the controls would have been most interesting In a study of the response to psychological stress in persons who were potentially hypertensives, Harris *et al* 1953 (81) demonstrated that the prehypertensives were less adaptable and more likely to get repetitive increase in blood pressure These might be of pathogenetic importance in the long run and lead to permanent hypertension

The studies during muscular exercise, however, clearly demonstrated that the peripheral vascular resistance was also involved in early essential hypertension

When the blood flow was high, particularly in the skeletal muscles, the resistance was clearly increased in the hypertensive men, already in their twenties It may therefore be argued that even if the CO was elevated in early essential hypertension at rest, and the total peripheral resistance was not significantly higher than in the controls, the most important disturbances in the young hypertensives could be failure of the peripheral resistance vessels to dilate sufficiently in response to the high resting flow – as they do to the increased flow during exercise This represents the difference from subjects with other hyperkinetic syndromes – such as anemia and thyrotoxicosis In these subjects, the total peripheral resistance is *sub normal* (4, 5, 20, 26, 95, 96, 68), and consequently the MAP remains normal in spite of the high flow If we assume that regulating mechanisms in the body try to maintain a mean blood pressure around 80–100 mm Hg resting – then the major defect in the hypertensives could be a failure in the vasodilation mechanism just as well as disturbance of the heart pump function It seems most reasonable to *conclude that both the heart pump and the peripheral resistance vessels are involved in early essential hypertension*

These conclusions are based on hemodynamic measurements of the systemic circulation alone The use of the Poiseuille's formula for calculation of the TPR can be criticized The formula is valid for non-pulsatile, laminar flow in straight, rigid tubes – a condition quite different from the systemic circulation Furthermore, the total peripheral resistance index does not reveal whether the vascular changes are general or only localised in some vascular areas What have more direct studies of the regional resistance in hypertensives in various ages and stages shown?

Unfortunately, as stated by Shepherd (142) this is a very complex field in which there is no unanimous opinion either on the importance of the sympathetic nervous system, or on the importance of local changes in the reactivity of the vessels to chemical stimuli, or of physical changes in the structure of the vessel wall In a study of the biophysical properties of the arteries in normotensive and hypertensive humans, Green *et al* (70) demonstrated a reduced distensibility in a segment of the brachial artery in hypertensives Increased neurogenic tone was found to be at least partially responsible for this

However, the common view is that the

resistance in the larger arteries is of relatively little importance for the total peripheral resistance, and that functional or morphological properties of the arterioles are most important (1, 25, 34, 35 55 103, 142)

Some studies have shown a disturbed function of the arterioles in essential hypertension Ashton (6) found an increased closing pressure which could be normalized by nerve blockade and by warming Mendlowitz *et al* (115) have found an increased reactivity to norepinephrine and Hinke (88) demonstrated a similar hyperresponsiveness without morphological changes in experimental hypertension Thus a functional abnormality of the arterioles which did not permit the same degree of dilation, could be responsible *for the observed reactions in this study* Some workers however, have found direct or indirect evidence for structural changes in the arterioles The great problem with histological studies is that the measurement of the arteriolar lumen and wall thickness in collapsed arterioles is very difficult By means of a new injection technique, Short (143) has recently described a decreased arteriolar diameter during maximal vasodilation in a post mortem study of the intestinal vessels in a small group of patients who had suffered from severe essential hypertension Such studies in young subjects with essential hypertension who have died by accident would be very interesting but of course very difficult to perform Many years ago Keith *et al* (103) studied the arterioles in biopsies from the pectoral muscle in subjects with essential hypertension *In contrast* to severe cases they rarely found increased wall thickness and decreased lumen in mild cases However such studies are difficult to interpret because a very slight reduction of the diameter of the arterioles will greatly increase the resistance which is inversely proportional to the fourth power of the radius e g 10% reduction of the internal radius increases the resistance to flow almost 50% (35) Therefore the application of histological *techniques to detect minor vessel changes* – changes which might have great functional importance must a priori be very difficult

In a plethysmographic study of the flow and resistance in the forearm in 131 untreated hypertensives and 50 normotensives Conway (35) made the following observations in subjects with mild hypertension (most of whom had essential hypertension) the flow at rest was greater than in the controls and the resistance was normal In subjects with severe hyper*tension the flow was normal and the re*sistance high During maximal vasodilation (by a combination of arm exercise and ischemia) the resistance was greater than in the controls even in the youngest group The flow however was still higher in the youngest group and subnormal in the oldest The observations on the TPRI in the present study at rest and during exercise thus fit well with Conway s results at rest it is not possible to demonstrate an abnormal total peripheral or muscular resistance in subjects with early hypertension but during high flow rates the resistance is clearly elevated in all groups of hypertensives including those with mild and early hypertension The observations in the present study may thus support the assumption of a reduced distensibility of the arterioles in the muscles *evident at maximal or submaximal flow*

A simultaneous study of the central and regional blood flow at rest supine has been performed by Brod *et al* (27 28) They found that the TPRI was elevated in subjects with essential hypertension and so

was the regional vascular resistance in the kidneys and the skin but the resistance in the resting muscles was probably reduced The findings, however, give no indication of the situation during work

A possible explanation of the histological findings by Short (143), the observations in the forearm by Conway (35) and the findings in this study is that, in early essential hypertension, the diameter of the arterioles is not reduced but their ability to dilate is restricted This is clearly revealed during muscular exercise when great dilation of arterioles in the working muscles is required As the years go by, the increased resting blood flow causes hypertrophy of the muscles in the arteriolar walls, and the resistance increases, mainly in areas usually receiving a high blood flow at rest

It is thus clear that the circulatory disturbances in early essential hypertension are *complex* and *both the heart and the peripheral vessels are involved*

This study does not give any definite answer to the question of whether essential hypertension *begins* with a disturbance of the heart pump in association with a normal peripheral vascular bed However, the study has demonstrated that a high total peripheral resistance is certainly not the only hemodynamic disturbance responsible for the high blood pressure in essential hypertension The cardiac factor is also important – particularly in young subjects, at least in men

VI Summary

I

The etiology of the most common type of high systemic arterial blood pressure – essential hypertension – is unknown, and there are large gaps in our knowledge of the pathogenesis of the disorder. High systemic arterial blood pressure may be established by a high cardiac output, a high total peripheral resistance or by a combination of both factors. While it is well documented that an increased total peripheral resistance is responsible for the high blood pressure in the late and advanced stages of essential hypertension, it is not clear if this is so at the beginning of the disorder.

It has been suggested that essential hypertension might begin with functional disturbances of the heart leading to an increased cardiac output and that the increased resistance might be a secondary phenomenon. There are few – if any – studies of unselected subjects with essential hypertension in early and later stages which elucidate the problem.

The object of the present investigation is to elucidate whether the hemodynamic mechanism behind elevated systemic arterial pressure in subjects with essential hypertension in the early phase is characterized by a high cardiac output and a normal peripheral resistance, in contrast to later stages where high resistance and normal or subnormal flow is expected. The hemodynamic pattern was studied at rest and also under the influence of stimuli to the circulatory system: 1) muscular exercise, 2) changes in the plasma volume and 3) a heavy meal. The purpose was to see whether abnormalities in the cardiac or the vascular response, or both, could be demonstrated.

II

The study was confined to men, 93 hypertensives and 48 normotensives. The upper limit for normotension was defined as casual blood pressure of 140/90 mm Hg according to WHO definition (7). The hypertensive subjects were drawn from a mass screening, from the files of two of the largest health centres in Bergen and from young subjects referred to the out-patient clinic at the hospital at the time of the study. The diagnostic criterias for essential hypertension are described. The patients were classified according the WHO criteria (7). The normotensive controls were healthy volunteers.

III

Practically all subjects were examined ambulatory. Great care was taken to try to remove any possible nervous tension during the experiments, and to keep a quiet atmosphere in the laboratory. No sedatives were given. Thin polyethylene

catheters were placed in the superior vena cava and the brachial artery Oxygen consumption, heart rate, cardiac output and intraarterial (brachial artery) and central venous pressure were recorded To test the reliability of the instrumental set up involved in the dye dilution method used for measurement of the cardiac output, model experiments were performed They documented a very high correlation between true flow and flow determined by the dye dilution method within a wide range of flow rates

The results were fed into an IBM digital computor for statistical calculations

The problem of comparing the hemodynamics in small and large subjects is described and the reasons for the use of index values in the presentation of the results are given

IV

A) All (141) subjects were studied *at rest*, undisturbed, 110 in the sitting position and 31 supine

The results showed that in the youngest hypertensive group (17–29 years), the peripheral resistance was not significantly different from that in the controls and the cardiac index tended to be high – the difference being almost significant The high cardiac output was maintained by a high heart rate and associated with an increase in the oxygen consumption

In the hypertensives over 30 years, the total peripheral resistance was significantly higher than in the controls, and the cardiac index was no different from that in the controls in subjects under 50 years In subjects over 50 years, most of whom had complications the cardiac index was low In the findings at rest, there was a trend pointing towards a change in the hemodynamics in essential hypertension from a ' high flow normal resistance ' pattern in the youngest via a ' high flow-high resistance" and a "normal flow high resistance" pattern in subjects with uncomplicated hypertension aged 30–50 years – to a ' low flow – high resistance pattern" in subjects over 50 years, where complications were frequently found The resistance pattern at rest seems to be quite constant for the individual as judged from re examination results after 1–3 months

B) The cardiovascular effect of graded muscular exercise (300, 600 and 900 kpm/min for 8–9 minutes) on an ergometer bicycle was studied in 68 hypertensives aged 17–56 years and in 33 normotensive controls aged 19–49 years The habitual physical activity in the groups was largely the same

The main purpose of this study was to see if the resistance of the arterial bed would be increased in the youngest hypertensives as well as in the oldest when the flow was greatly increased, and to study whether or not the cardiac response to exercise was normal in hypertensives The results demonstrated that, during muscular work, already at a low exercise level, the total peripheral resistance index was significantly *higher* than in the controls in the hypertensives at *all ages, even in men in their twenties* The findings are consistent with the assumption of a reduced vasodilating capacity in the muscles in hypertension In *all* ages, the cardiac output in relation to the oxygen uptake tended to be *lower* in the hypertensives and the arterio venous difference higher The difference from the controls increased with work load and aging The stroke volume during work was lower in the hypertensives in all ages The work of the heart tended to be higher than in the controls at low work levels but at high work levels, the difference from the controls was not significant owing to a low flow factor in the pressure-

flow product The left ventricular stroke work was not significantly different from that in the controls owing to a low stroke volume The results and the possibility of the increased resistance being responsible for the cardiac changes during work – in contrast to similar functional cardiac changes during heart failure – are discussed

C) The cardiovascular effect of changes in the plasma volume were studied in 10 normotensives and 18 hypertensives at rest The main purpose of the study was to see if it were possible to demonstrate an increased cardiac response in early essential hypertension – suggestive of a disturbed regulation of the heart pump or of a reduced venous capacity leading to a grater increase in the central venous pressure 500 + 500 ml of dextran was infused and thereafter 500 ml blood was removed and the hemodynamic parameters were measured before and after each change in plasma volume

The increase in the cardiac index after the infusions tended to be *higher* in hypertensives < 40 years than > 40 years and higher than in the controls < 40 years This is suggestive of a disturbed autonomous regulation of the heart pump in early essential hypertension but the difference did not reach statistical significance

The results revealed no abnormalities on the venous side of the circulation either in the young subjects with mild hypertension or in the older hypertensives The artificial increase in the cardiac output did not demonstrate any reduced conductance of the arterial vascular system either in young or in older hypertensives It is emphasized that the results are less unanimous than those obtained during exercise and great care is necessary in the interpretation of the results

D) A pilot study was made of the hemodynamic effects of a heavy meal in 7 hypertensives at various stages and in 5 controls The main intention was to try to create a regional distribution of the blood flow different from that during work and to increase mainly the *renohepatosplanchnic* blood flow As it has been suggested that the total peripheral resistance in this vascular area is increased early in the course of essential hypertension it was thought to be of interest to see whether it was possible to demonstrate a reduced conductance of the arterial bed by this experiment

The results showed a similar increase in the cardiac output and a similar reduction of the total peripheral resistance index in hypertensives and controls The experiment was meant to be a pilot study and the small number of subjects studied necessitate care in the interpretation of the results

V

The importance of a carefully selected and well defined patient group together with suitable controls for the study of the hemodynamic changes in essential hypertension is pointed out

The hemodynamic findings are compared with results obtained by other methods It is not possible to tell from the present study whether or not a disturbed function of the heart is the *primary disorder in essential hypertension* It is stated that, at least in young men with what we today call essential hypertension, an elevated total peripheral resistance at rest is not common, but a high cardiac pump function is a characteristic feature

The findings during muscular exercise however, demonstrate that even in men in their twenties the vascular resistance *is increased* It is concluded that the hemodynamic disturbances are complex, and that *both* the heart pump and the peripheral vessels are involved in essential hypertension in the early phase

VII Appendix

Comparison of the stroke volume response to exercise on to different types of ergometer bicycles (saddle seat type and armchair type)

The stroke volume during exercise tended to be somewhat higher than reported by other investigators who had used an ergo meter with a saddle seat (99, 135, 136) The model experiments gave no indication of methodological over-estimation of the cardiac output, and the heart rate was obtained by ECG and should obviously not involve any greater error than about 1% On the ergometer used in this study, the subject exercises with his lower extremities nearly horizontal, in contrast to an ordinary saddle seat ergometer which is usually adjusted so that the subject can extend his knee and the body position is then nearly vertical These differences might be of importance for the venous return and the stroke volume response (76)

It was therefore decided to examine these two different types of ergometers and see whether they gave different stroke volume responses

Material and methods

Five healthy normotensive males aged 20–34 years were examined Body weight ranged from 71–80 kg mean 77 4 kg BSA 1 90–2 01 m² mean 1 97 m² Four had medium and one high physical activity The methods and experimental procedure in subjects 1,2 and 3 were exactly as described in chapter III After the three work tests on the arm chair ergometer had been performed, the subjects rested for 20–30 minutes and then exercised at 300 and then 600 kpm/min – and one subject also at 900 kpm – on an ordinary ergo meter bicycle with a saddle seat which was adjusted to allow full extension of the knee during cycling The subjects rested for about 10 minutes in a chair between the work tests

In subjects 4 and 5 the sequence of the ergometers was reversed In these two subjects all 3 loads were carried out on both ergometers

Results

The mean oxygen uptake at the three work loads on the arm-chair ergometer was 464 8 770 6 and 1202 2 ml/min/m² – quite close to the mean values for the normotensives aged 17–39 years The oxygen uptake during the work loads on the saddle seat ergometer, tended to be a little higher – the mean values being 574 8, 916 4 and 1265 6 ml/min/m² The mean heart rate at the three work loads on the arm-chair ergometer was 100 6, 130 6 and 165 6 beats/min – somewhat higher than for the total normotensive group aged 19–39 years (Only one of the

Table 42 *Comparison of the stroke volumes in five subjects during work on two types of ergometer bicycles Stroke index (ml/stroke/m²)*

Load	Arm chair type			Saddle seat type			Difference chair saddle		
	300	600	900	300	600	900	300	600	900
1	71	71	72	59	60	57	−12	−11	−15
2	75	86	78	57	67	—	−18	−19	—
3	77	[illegible]	65	57	62	—	−20	−20	—
4	78	90	93	87	87	77	+9	−3	−16
5	66	77	66	68	66	56	+2	−11	−10
Mean	73 4	81 2	74 8	65 6	68 4	63 3	−7 8	−12 8	−13 7

1 2 and 3 First chair – then saddle 4 and 5 First saddle – then chair

subjects had high physical activity level) The mean heart rates on the saddle seat ergometer were 116 4, 149 2 and 173 0 beats/min The stroke index values are shown in table 42 It is seen that with two exceptions, the stroke index on the arm chair ergometer was higher than at the corresponding work load on the saddle seat ergometer On both ergometers, the SI tended to be highest at the 600 kpm/min load, but, by and large the mean values at the three loads showed no great changes On average the SI during work was about 10 ml/stroke/m² (or the SV about 20 ml/stroke) higher on the arm chair ergometer than on the saddle seat ergometer

Discussion and conclusion

The results demonstrate that the body position is of importance for the stroke volume response to exercise It is well known that the SV in resting subjects is lower in the upright position than supine (17 32 160 161) The differences are thought to be due to the differences in the venous return, which again is influenced by gravity (161) On a saddle ergometer fainting episodes are not rare if the subject is resting (17 45, 135) and such episodes probably represent pooling of blood in the lower extremities and drastic fall in the venous return leading to great decrease in cardiac output

When the subject works, the muscle pump" will counteract the influence of gravity on the venous return (76) The present results showing a lower SV during work in a nearly vertical body position on a saddle seat ergometer than on the arm chair type with the lower extremities more horizontal, can most probably be explained by the influence of the body position on the venous return during work also In other words the muscle pump is not efficient enough in the vertical position to counteract the influence of gravity completely

The results demonstrate that the rather high stroke index values in the present study may be explained at least partially, by the body position during work The results cannot therefore be compared directly with those obtained by other investigators using an ordinary ergometer with a saddle seat In healthy controls the stroke volume is about 20 ml higher than during work on an ordinary ergometer

References

1 ABRAMSON, D I & FIERST, S M Resting blood flow and peripheral vascular response in hypertensive subjects Amer Heart J *23* 84, 1942

2 AMERY, A, JULIUS, S, WHITLOCK L S & CONWAY, J Influence of hypertension on the hemodynamic response to exercise In press 1967

3 ANDERSEN K LANGE Respiratory recovery from muscular exercise of short duration Oslo University Press 1959

4 ANDRUS E C The thyroid and the circulation Circulation *7* 437, 1953

5 ANTHONISEN, P, HOLST, E & THOMSEN, AA CHR Determination of cardiac output and other hemodynamic data in patients with hyper and hypothyroidism using dye dilution technique Scand J clin Lab Invest *12* 472 1960

6 H ASHTON The minimum critical closing pressure of digital blood vessels in hypertension Clin Sci *28* 385, 1965

7 Arterial hypertension and ischaemic heart disease Preventive aspects Report of an Expert Committee Wld Hlth Org techn Rep Ser No 231 1962

8 ASMUSSEN E & NIELSEN M The cardiac output in rest and work determined simultaneously by the acethylene and the dye injection methods Acta physiol scand *27* 217, 1953

9 ASMUSSEN E & NIELSEN M Cardiac output during muscular work and its regulation Physiol Rev *35* 778 1955

10 AYMAN, D & GOLDSHINE A D The breath holding test A simple standard stimulus of blood pressure Arch intern Med *63* 899 1939

11 BALDWIN, D S, BIGGS, A V, GOLDRING, W, HULET, W H & CHASIS, H Exaggerated natriuresis in essential hypertension Amer J med *24* 893, 1958

12 BARATH E Arterial hypertension and physical work Arch intern Med *42* 297, 1928

13 BECHGAARD, P The natural history of benign hypertension *In* Essential hypertension An international symposium Ed by K L BOCK & P T COTTIER, Springer Verlag Berlin, 1960 p 198

14 BECKLAKE, M R, FRANK H DAGENAIS, G R, OSTIGUY, G L & GUZMAN, C A Influence of age and sex on exercise cardiac output J appl Physiol *20* 938, 1965

15 BELLO C T, SEVY, R W & HARAKAL, C Varying hemodynamic patterns in essential hypertension Amer J med Sci *250* 24, 1965

16 BELLO, C T, SEVY, R W, HARAKAL, C & HILLYER, P N Relationship between clinical severity of disease and hemodynamic patterns in essential hypertension Amer J med Sci *253* 92 1967

17 BEVEGÅRD S, HOLMGREN A & JONSSON B The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume Acta physiol scand *49* 279 1960

18 BIRCHALL, R TUTHILL, S W JACOBS, W S TRAUTMAN W J Jr & FINDLEY, T Renal excretion of water, sodium and chloride Circulation *7* 258 1953

19 BIRKE, G DUNÉR, H ELLER U S V & PLANTIN L O Studies on the adreno-

cortical adreno-medullary and adrenergic nerve activity in essential hypertension Z Vitamin- Hormon u Fermentforsch. 9 41, 1958

20 BISHOP, J M DONALD, K W & WADE O L. Circulatory dynamics at rest and on exercise in the hyperkinetic states Clin Sci 14 329 1955

21 BJERKEDAL, T & NATVIG, H Changes in blood pressure with age Acta med scand 180 257, 1966

22 BOLOMEY A A MICHIE A. J MICHIE C. BREED E S SCHREINER G E. & LAUSON H D Simultaneous measurement of effective renal blood flow and cardiac output in resting normal subjects and patients with essential hypertension J clin Invest 28 10 1949

23 BOUSVAROS G A PALMER, W H. SEKELJ P & Mc. GREGOR M Comparison of central and peripheral injection sites in the estimation of cardiac output by dye dilution curves Circulat. Res 12 317 1963

24 BRANDFONBRENER M LANDOWNE M & SHOCK N W Changes in cardiac output with age Circulation 12 557 1955

25 BREST A. N Hemodynamic response to antihypertensive drug therapy J Amer med. Ass 192 127, 1965

26 BREWSTER W R JR, ISAACS J P OSGOOD R F & KING T L. The hemodynamic and metabolic interrelationship in the activity of epinephrine norepinephrine and the thyroid hormones Circulation 13 1 1956

27 BROD J Essential hypertension Hæmodynamic observations with a bearing on its pathogenesis Lancet II 773 1960

28 BROD J Hemodynamic basis of acute pressor reaction and hypertension Brit Heart J 25 227 1963

29 BRUCE R A. COBB L. A., MORLEDGE J H & KATSURA, S Effects of posture upright exercise and myocardial stimulation on cardiac output in patients with deseases affecting diastolic filling and effective systolic ejection of left ventricle Amer Heart J 61 476 1961

30 BRUCE TH A & SHILLINGFORD J P The normal resting cardiac output Serial determinations by a dye dilution method. Brit Heart J 24 69 1962

31 CAMPIONE K M ANDAY I J SERRATO, M & EARLE D P Cardiac index in ambulatory patients estimated by precordial dilution curves Amer J Cardiol 7 779 1961

32 CHAPMAN C B FISHER J A & SPROULE B J Behaviour of stroke volume at rest and during exercise in human beings J clin Invest 39 1208 1960

33 CONN J W COHEN E L. ROVNER D R & NESBIT R M Normokalemic primary aldosteronism J Amer med Ass 193 200 1965

34 CONWAY J The nature of the increased peripheral resistance in hypertension Amer J Cardiol 5 649 1960

35 CONWAY J A vascular abnormality in hypertension A study of blood flow in the forearm Circulation 27 520 1963

36 CORA, D DEBIASI S MAGGIA A & CORTESI S The circulating blood volume as a factor regulating salt excretion in man Clin Sci. 22 239 1962

37 COTTIER P WELLER J M & HOOBLER S W Sodium chloride excretion following salt loading in hypertensive subjects Circulation 18 196 1958

38 DONALD K W BISHOP J M CUMMINGS, G & WADE O L. Effect of exercise on cardiac output and circulatory dynamics of normal subjects Clin Sci. 14 37 1955

39 DUNCAN C. H STEVENSON J P & WOLFF H. G Life situations emotions and exercise tolerance Psychosom. Med 13 36 1951

40 Edit. Circulation in essential hypertension Lancet. II 1323 1964

41 EICH R. H PETERS, R J CUDDY R P SMULYAN H & LYONS R. H The hemodynamics in labile hypertension Amer Heart J 63 188 1962

42 EICH R H CUDDY R. P SMULYAN H. & LYONS R. H Hemodynamics in la

bile hypertension A follow up study Circulation *34* 299, 1966

43 EIFF, A W v, JESDINSKY, H J, JORGENS H & MAETZEL, F K Zur Frage des Einflusses von Geschlecht, Alter und Blutdruck auf Stress-Reaktionen von Hochdruckkranken *In* Verhandlungen der Deutschen Gesellschaft fur Kreislaufforschung 28 Tagung Thema Essentielle Hypertonie Ed by R Thauer & C Albers Dr Dietrich Steinkopff Verlag, Darmstadt, 1963, p 286

44 EILERTSEN, E & HUMERFELT S The blood pressure in a representative population sample Acta med scand In press, 1967

45 EKELUND, L G & HOLMGREN, A Circulatory and respiratory adaption during long term, non-steady state exercise in the sitting position Acta physiol scand *62* 240 1964

46 ELIASH, H & VARNAUSKAS, E Hemodynamiska och kliniska efterundersokningar på hypertonipasienter Skandinavisk hypertonimote 1965

47 ENESCU, V GEORGESCU M, & TEODORESCU, S Die Beziehungen zwischen den systematischen hamodynamischen Storungen und den klinischen Verlaufsstadien der Hochdruckkrankheit Z Kreisl Forsch *50* 986, 1961

48 EPSTEIN S E, BARER, G D, STAMPFER M, ROBINSON, B F & BRAUNWALD E Characterization of the circulatory response to maximal upright exercise in normal subjects and patients with heart disease In press 1967

49 EULER, U S v Noradrenaline Charles C Thomas Springfield III 1956

50 EVANS, G L, SMULYAN, H & FICH R Role of the peripheral resistance in the control of the cardiac output Circulation *34* *Suppl III* 99 1966

51 FARNSWORTH E B Renal reabsorption of chloride and phosphate in normal subjects and in patients with essential hypertension J clin Invest *25* 897, 1946

52 FEJFAR Z & WIDIMSKY J Juvenile hypertension *In* The patogenesis of essential hypertension By Cort, J H, Fencl, V, Hejl, Z & Jirka J State medical publishing house Prague 1961, p 33

53 FINKIELMAN, S, WORCEL M & AGREST, A Hemodynamic patterns in essential hypertension Circulation *31* 356, 1965

54 FLOYER, M A The mechanism underlying the response of the hypertensive subject to a saline load Expansion Scientifique Francaise, Paris *I* 1966

55 FOLKOW, B, GRIMBY, G & THULESIUS, C Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta physiol scand *44* 255, 1958

56 FOSTER, G L & REEVES T J Hemodynamic responses to exercise in clinically normal middle aged men and in those with angina pectoris J clin Invest *43* 1758, 1964

57 FRASER, R S & CHAPMAN, C B Studies on effect of exercise on cardiovascular function, blood pressure and heart rate Circulation *9* 193 1954

58 FREIS F D Hemodynamics of hypertension Physiol Rev *40* 27, 1960

59 FRICK M H Observations on hemodynamic factors and left heart performance in essential hypotension Acta med scand *172* suppl 380, 1962

60 FRICK M H, KONTTINEN, A & SARAJAS, H S S Effects of physical training on circulation at rest and during exercise Amer J Cardiol *12* 142, 1963

61 FROHLICH E D, ULRYCH, M, TARAZI R C, DUSTAN, H P & PAGE, I H A hemodynamic comparison of essential and renovascular hypertension Circulation *35* 289, 1967

62 FRYE R L & BRAUNWALD, E Studies on Starling s law of the heart I The circulatory response to acute hypervolemia and its modification by ganglionic blockade J clin Invest *39* 1043, 1960

63 GABE, I & SHILLINGFORD, J The photoelectrical earpiece technique for recording dye dilution curves Brit Heart J *23* 271 1961

64 GLAZER, G A A study of some haemodynamic parameters in essential hypertension Cor et vasa (Praha) *5* 165 1963

65 GOLDRING W & CHASIS H Hypertension and hypertensive disease The Commonwealth Fund, New York 1944

66 GORLIN R BRACHFELD, N TURNER J D MESSER J V & SALAZAR, E The idiopathic high cardiac output state J clin Invest *38* 2144 1959

67 GLADSTONE S A Cardiac output and arterial hypertension Publ by author New York *33* 1935

68 GRAETTINGER, J S MUENSTER J J SELVERSTONE, L A & CAMPBELL J A A correlation of clinical and hemodynamic studies in patients with hyperthyroidism with and without congestive heart failure J clin Invest *38* 1316 1959

69 GRANATH A, JONSSON, B & STRANDELL T Circulation in healthy old men studied by right heart catheterization at rest and during exercise in supine and sitting position Acta med scand *176* 425 1964

70 GREENE M A FRIEDLANDER R BOLTAX A J, HADJIGEORGE C & CUSTIO G A Biophysical properties of arteries in human hypertension Circulation *30* suppl *III* 89 1964

71 GREEN H D, SCHMID, H E & RAPELA C E Autoregulation in vascular beds Possible role of autoregulation in the increased resistance to flow in hypertension Hypertension *13* 144 1965

72 GRIMBY G Exercise in man during pyrogen induced fever Scand J clin Lab Invest *14* *Suppl 67* 1962

73 GRIMBY G, NILSSON N J & SALTIN B Cardiac output during submaximal exercise in active middel aged athletes J appl Physiol *21* 1150 1966

74 GRIMBY G NILSSON N J & SANNE H Several determinations of cardiac output at rest Brit Heart J *28* 118 1966

75 GRIMBY G HAGGENDAL E & SALTIN B 133Xe-clearance från lårmuskulatur under arbete Nord Med *76* 1069 1966

76 GUYTON A G Cardiac output and its regulation W B Saunders Company Philadelphia 1963

77 HAMILTON W F & REMINGTON, J W Some factors in the regulation of the stroke volume Amer J Physiol *153* 287 1948

78 HAMMERSTEN J F HELLER B I & EBERT I The effects of dextran in normovolemic and oligemic subjects J clin Invest *32* 340 1953

79 HANENSON I B TAUSSKY H H POLASKY N RANSOHOFF W & MILLER B Renal excretion of sodium in arterial hypertension Circulation *20* 498 1959

80 HANSON J S TABAKIN B S & LEVY A M Comparison of cardio-respiratory responses to graded upright exercise in normal men aged 20–29 and 30–39 Brit Heart J *28* 557 1966

81 HARRIS R E SOKOLOW M CARPENTER L G JR FREEDMAN M & HUNT S P Response to psychologic stress in persons who are potentially hypertensive Circulation *7* 874 1953

82 HARRIS W S SCHOENFELD C D GWYNNE I H WEISSLER A M & WARREN J Observations on the cardiovascular response to fear and anger Circulation *30* *III*-92 1964

83 HARVEY, R M, SMITH W M PARKER J O & FERRER M I The response of the abnormal heart to exercise Circulation *26* 341 1962

84 HEGGLIN R RUTISHAUSER W, KAUFMANN G LUTHY E & SCHEU H Kreislaufdiagnostik mit der Farbstoffverdunnungsmethode Georg Thieme Verlag Stuttgart 1962

85 HERMANSEN L Aerob arbeidskapasitet i relasjon til alder og kjønn Hovedfagsoppgave i zoofysiologi Oslo universitet 1964

86 HICKAM J B CARGILL W H & GOLDEN A Cardiovascular reactions to emotional stimuli Effect on the cardiac output arteriovenous oxygen difference arterial pressure and peripheral resistance J clin Invest *27* 290 1948

87 HINES E A & BROWN G E A standard

stimulus for measuring vasomotor reactions Its application in the study of hypertension Proc Mayo clin *7* 332 1932

88 Hinke, J A M In vitro demonstration of vascular hyperresponsiveness in experimental hypertension Circulat Res *17* 359 1965

89 Holland W W & Humerfelt, S Measurement of blood pressure Comparison of intraarterial and cuff values Brit med J *II* 1241 1964

90 Hollander W & Judson W L Electrolyte and water excretion in arterial hypertension I Studies in non medically treated subjects with essential hypertension J clin Invest *36* 1460 1957

91 Holmgren A Circulatory changes during muscular work in man With special reference to arterial and central venous pressure in the systemic circulation Scan J clin Lab Invest *8* suppl 24 1956

92 Hoobler, S W Current concepts of the mechanism of essential hypertension In Brest A N & Moyer J H Hypertension Recent advances Lea & Febiger, Philadelphia 1961

93 Horrobin F A theory of hypertension Lancet *I* 576 1966

94 Housel, E L Clinical significance and consequences of labile hypertension Amer J Cardiol *17* 617, 1966

95 Howitt, G & Rowlands D J The heart in hyperthyroidism Amer Heart J *73* 282 1967

96 Humerfelt S Muller O & Storstein O The circulation in hyperthyroidism a cardiac catherization study before and after treatment Amer Heart J *56* 87 1958

97 Judson W F Hollander W & Wilkins R W The effect of exercise in the supine position on cardiovascular and renal function in hypertensive patients before and during chronic oral hydralazine therapy J Lab clin Med *49* 672 1957

98 Julius S Harburg E McGinn N F Keyes J & Hoobler S W Relation between casual blood pressure readings in youth and at age 40 J chron Dis *17* 397, 1964

99 Julius, S, Amery A Whitlock L S & Conway J Influence of age on the hemodynamic response to exercise In press 1967

100 Karlefors T Nielsen R & Westling, H On the accuracy of indirect auscultatory blood pressure measurement during exercise Acta med scand *180* suppl 449 91 1966

101 Knutsen B Lund Johansen P & Hatletveit E A linearily responding monochromatic cuvette densitometer for indocyanine green Scand J clin Lab Invest *18* 673 1966

102 Knutsen B Unpublished results 1966

103 Keith N M Wagener H P & Barker, N W Some different types of essential hypertension their course and prognosis Amer J med Sci *197* 332 1939

104 Kuhns K & Brahms O Die Prognose der essentiellen Hypertonie Dr Dietrich Steinkopff Verlag Darmstadt 1964

105 Konig K Reindell H Steim H & Messhoff K Beitrag zur Hamodynamik hypertoner Regulationsstorungen Z Kreisl Forsch *48* 923 1959

106 Laragh J Recent advances in Hypertension Amer J Med *39* 616 1965

107 Laragh J Renin and aldosterone in the diagnosis of some forms of hypertension Read at Amer Heart Assn 39th scientific session, New York 1966

108 Ledingham J M & Cohen R D Circulatory changes during the reversal of experimental hypertension Clin Sci *22* 69 1962

109 Ledingham J M *Circulatory changes* in experimental renal hypertension International club on arterial hypertension *I* 393 1966

110 Lund-Johansen P Det hamodynamiska monstret vid essentiell hypertoni i vila och under arbete Discussion at Skandinaviskt Hypertonimote Marstrand 1965

111 Lund-Johansen P Thorsen T & Stoa K F A comparison of two meth

ods for measurement of aldosterone secretion rate in man Acta Endocrin *53* 177, 1966

112 LUND-JOHANSEN P, THORSEN T & STOA K F Aldosteron og hypertensjon Nord Med In press 1967

113 MALMBORG, R O A clinical and hemodynamic analysis of factors limiting the cardiac performance in patients with coronary heart disease Acta med scand *177* suppl 426 1965

114 McMICHAEL J & SHARPY-SCHAFER E P Cardiac output in man by direct Fick method Effects of posture venous pressure change atropine and adrenaline Brit Heart J *6* 35, 1944

115 MENDLOWITZ, M, GITLOW S WOLF R & NAFTCHI N E. On the intergration of factors in essential hypertension Amer Heart J *67* 397 1964

116 MIASNIKOV, A L. The significance of disturbances of higher nervous activity in the pathogenesis of hypertensive disease In Cort, J H Fencl V Hejl W & Jirka J State Medical Publishing House Prague 1961

117 MOORE, J W, KINSMAN J M HAMILTON W F & SPURLING R G Cardiac output determinations Comparison of the injection method with the direct Fick procedure Amer J Physiol *89* 331 1929

118 MULLER C Cardiopulmonary hemodynamics of chronic lung disease Oslo University Press 1959

119 NAEYE R L. Arteriolar abnormalities with chronic systemic hypertension A quantitative study Circulation *35* 662 1967

120 NAGLE F J NAUGHTON J & BALKE, B Comparison of direct and indirect blood pressure with pressure flow dynamics during exercise J appl Physiol *21* 317 1966

121 OFSTAD J, LUND-JOHANSEN P & KOLSAKER L Dye dilution measurements of renal blood flow in vivo and in glass models Scand. J clin Lab Invest In press 1967

122 OLMSTEDT F & PAGE J H Hemodynamic changes in trained dogs during experimental renal hypertension Circulat Res *16* 134 1965

123 PAPPER S BELSKY J L & BLEIFER K H The response to administration of an isotonic sodium chloride lactate solution J clin Invest *39* 876 1960

124 PETERSON L H Hemodynamic alterations in essential hypertension In A N Brest & J H Meyer Hypertension Recent Advances Lea & Febiger Philadelphia 1961

125 PICKERING G W High blood pressure J & A Churchill Ltd London 1955

126 PICKERING, G W Inheritance of high blood pressure In Bock K D & Cottier P T Essential hypertension Springer Verlag Berlin 1960 p 30

127 PICKERING G W The nature of essential hypertension J & A Churchill Ltd London 1961

128 PLATT R The nature of essential hypertension In Bock K D & Cottier P T Essential hypertension Springer Verlag Berlin 1960 p 39

129 RADIGAN L R & ROBINSON S Effects of environmental heat stress and exercise on renal blood flow and filtration rate J appl Physiol *2* 185 1949

130 REEVES J T GROVER R F BLOUNT S G & FILLEY G F Cardiac output response to standing and treadmill walking J appl Physiol *16* 283 1961

131 ROWE G G CASTILLO C A MAXWELL, G M & CRUMPTON C W A hemodynamic study of hypertension including observations on coronary blood flow Ann intern Med. *54* 405 1961

132 ROWELL L.B BLACKMON J R & BRUCE R A Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man J clin Invest *43* 1677 1964

133 SANNERSTEDT R & WERKO L Hemodynamic aspects of modern medical treatment of arterial hypertension Med. Clin N Amer *46* 1639, 1962

134 SANNERSTEDT, R & VARNAUSKAS E

Total peripheral resistance during muscular exercise in arterial hypertension IV Congressus Cardiologicus Europeaeus Abstracta Prague 1964

135 SANNERSTEDT, R Hemodynamic response to exercise in patients with arterial hypertension Acta med scand *180* suppl 458 1966

136 SALTIN B Aerobic work capacity and circulation at exercise in man Acta physiol scand *62* suppl 230, 1964

137 SCHNABEL, T G ELIASCH H THOMASSEN, B & WERKO, L The effect of experimentally induced hypervolemia on cardiac function in normal subjects and patients with mitral stenosis J clin Invest *38* 117 1959

138 SCHOLANDER, P F Analyzer for accurate estimation of respiratory gases in one half cubic centimeter samples J biol Chem *167* 235 1947

139 SELDINGER S I Catheter replacement of the needle in percutaneous arteriography A new technique Acta radiol (Stockh) *39* 368 1953

140 SELZER A & McCAUGHEY D J Hemodynamic patterns in chronic cardiac failure Amer J Med *28* 337 1960

141 SHAPIRO A P An experimental study of comparative responses of blood pressure to different noxious stimuli J chron Dis *13* 293 1961

142 SHEPHERD, J Physiology of the circulation in human limbs in health and disease W B Saunders Comp London 1963

143 SHORT, D Morphology of the intestinal arterioles in chronic human hypertension Brit Heart J *28* 184 1966

144 SIMONSON E & BROZEK J Russian research on arterial hypertension Ann intern Med *50* 129 1959

145 SJOERDSMA A Relationships between alterations in amine metabolism and blood pressure Circulat Res *9* 734 1961

146 SMULYAN H VINCENT, W A KASHEMSANT L GUDIN R P & EICH R H Evaluation of the cardiac index Amer Heart J *72* 621 1966

147 SNYDER, D, BREST, A N & NOVACK P Systolic hypertension A presenting finding in the hyperkinetic heart syndrome (P) Circulation *32*, *suppl 2* 199, 1965

148 SOKOLOW, M & LYON, T P The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads Amer Heart J *37* 161, 1949

149 SOKOLOW M & HARRIS, R E The natural history of hypertensive disease In Brest, A N & Moyer J H Hypertension Recent Advances Lea & Febiger Philadelphia 1961, p 8

150 STARLING, E H The Linacre lecture on the law of the heart Longham Green & Co London 1918

151 STEAD, E A JR, WARREN J V, MERRILL A J & BRANNON, E S The cardiac output in male subjects as measured by the technique of right atrial catheterization Normal values with observations on the effect of anxiety and tilting J clin Invest *24* 326, 1945

152 STENBERG J ÅSTRAND, P O EKBLOM B, ROYCE J & SALTIN, B Hemodynamic response to work with different muscle groups sitting and supine J appl Physiol *22* 61, 1967

153 STROM G Pers communication 1964

154 TABAKIN B S, HANSON, J S MERRIAM T W & CALDWELL, E J Hemodynamic response of normal men to graded tread mill exercise J appl Physiol *19* 457, 1964

155 TAYLOR S H, DONALD K W & BISHOP, J M Circulatory studies in hypertensive patients at rest and during exercise With a note on the Starling relationship in the left ventricle in essential hypertension Clin Sci *16* 351, 1957

156 ULLMANN, T D & CZACZKES, W J Increased sensitivity of a volume regulating mechanism in the hypertensive state Arch fur Kreislaufforsch *33* 137, 1960

157 ULRYCH M HOFMAN, J & HEJL Z Cardiac and renal hyperresponsiveness to acute plasma volume expansion in hypertension Amer Heart J *68* 193, 1964

158 [illegible] E. Studies in [illegible] cardiovascular disease with [illegible] reference to cardiac function. Scand. J. clin. Lab. Invest. [illegible]

159 Wade, O. L. & Bishop, J. M. Cardiac output and regional blood flow. Blackwell Scientific Publications, Oxford, [illegible]

160 Wang, Y., Marshall, R. J. & [illegible] Z. T. The effect of changes in posture and of graded exercise on stroke volume in man. J. clin. Invest. 39 [illegible]

161 Ward, R. J., Danziger, F., [illegible] J. J., Allen, G. D. & Tolas, A. G. Cardiovascular effects of change of posture. Aerospace Med. 37 [illegible]

162 Warren, J. V., Brannon, E. S., [illegible] E. A. & Merrill, A. J. The effect of venesection and the pooling of blood in the extremities on the atrial pressure and cardiac output in normal subjects with observations on acute circulatory collapse in three instances. J. clin. Invest. 24 337 1945

163 Weller, A. & Groom, J. J. Effect of a simple deconditioning procedure on the diuretic and natriuretic response of hypertensive patients to a hypertonic salt load. Circulation 35 260 1967

1[illegible] [illegible] L. & [illegible] E. Studies on the [illegible] in man. IV. Cardiac output and blood pressure in the [illegible] right ventricle and pulmonary [illegible] in patients with [illegible] cardiovascular disease. Acta med. scand. [illegible]

1[illegible] W[illegible] S., C[illegible] P. [illegible] E. M. & W[illegible] H. G. [illegible] and [illegible] Williams & Wilkins Co. Baltimore [illegible]

1[illegible] W[illegible] E. [illegible] und [illegible] beim [illegible] Fortschritte [illegible] dem Gebiete der [illegible] H. [illegible] Ed. by L. [illegible] & H. J. H[illegible] Georg Thieme Verlag, Stuttgart, 19[illegible] p. 1[illegible]

167 Åstrand, I. Aerobic work capacity in men and women with special reference to age. Acta physiol. scand. 49 suppl. 1 1960

168 Åstrand, P. O., Cuddy, T. E., Saltin, B. & Stenberg, J. Cardiac output during submaximal and maximal work. J. appl. Physiol. 19 268 1964

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SUPPLEMENTUM 483

CYTODIAGNOSIS OF THYROIDITIS

A COMPARATIVE STUDY OF CYTOLOGICAL, HISTOLOGICAL, IMMUNOLOGICAL AND CLINICAL FINDINGS IN THYROIDITIS PARTICULARLY IN DIFFUSE LYMPHOID THYROIDITIS

BY

P SIGVARD PERSSON

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SYDSVENSKA DAGBLADETS AKTIEBOLAG
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To my wife and children

CONTENTS

CHAPTER I
INTRODUCTION

CLASSIFICATION AND NOMENCLATURE OF THYROIDITIS

Since the original description of Riedel's struma (162), de Quervain's thyroiditis (159) and Hashimoto's struma (91) opinions have differed on the relationship between these types of thyroiditis (for references see 75, 124). The nomenclature has also become bewildering, one and the same type of thyroiditis being described by different names. But it now appears to be generally agreed that acute suppurative, de Quervain's, Riedel's and Hashimoto's thyroiditis are four different entities and the first three clinicopathologically well defined diseases (35, 68, 92, 93, 124, 226). On the other hand, opinions still differ regarding the relation of Hashimoto's thyroiditis with, and its differentiation from, chronic non-specific thyroiditis, focal thyroiditis and primary myxoedema. The classical clinical picture of Hashimoto's disease is well defined (e.g. 68, 107) but embraces only a small proportion of the histologically verified cases (35, 124). The histological definition of Hashimoto's thyroiditis varies from author to author (48, 132, 148). MASI et al. (132) had 7 pathologists examine the same sections from 16 cases of thyroiditis initially classified as Hashimoto's thyroiditis or chronic non-specific thyroiditis and found such overlapping between the diagnoses made by these pathologists that they concluded that Hashimoto's thyroiditis and chronic non-specific thyroiditis can hardly be regarded as histo-pathologically different conditions. It has been claimed from various quarters that the differences between Hashimoto's thyroiditis, chronic non-specific thyroiditis and focal thyroiditis are differences in degree rather than in kind (76, 124, 212, 228).

The morphological and clinical similarities between Hashimoto's thyroiditis and primary myxoedema have also been described (24, 189, 222). There is thus reason to regard focal thyroiditis, chronic non-specific thyroiditis, Hashimoto's thyroiditis and primary myxoedema as different phases of one and the same disease. After thyroid antibodies had been demonstrated in these conditions the blanket name 'autoimmune thyroiditis' has often been used. In recent years three morphological subgroups have been suggested, *viz.* focal, diffuse and atrophic lymphoid thyroiditis (48,

94, 178). Atrophic lymphoid thyroiditis is the morphological basis of primary myxoedema. Since there is probably a gradual transition between focal and diffuse lymphoid thyroiditis, distinction between these two forms may appear artificial, but is well justified from a clinical point of view.

In this presentation the following classification and nomenclature of thyroiditis will be used. Synonyms are given in brackets. Thyroiditis due to trauma, tuberculosis or radiation will not be considered.

1. Acute suppurative thyroiditis.
2. Subacute thyroiditis (de Quervain's thyroiditis, granulomatous thyroiditis, pseudotuberculous thyroiditis, giant cell thyroiditis, creeping thyroiditis, struma granulomatosa, struma fibrosa—giant cell variant, acute non-infectious thyroiditis and acute non-suppurative thyroiditis).
3. Lymphoid thyroiditis.
   - A. Diffuse lymphoid thyroiditis (Hashimoto's struma, Hashimoto's thyroiditis, Hashimoto's disease, struma lymphomatosa, lymphadenoid goitre, chronic lymphoid thyroiditis, lymphocytic thyroiditis, lymphoid thyroiditis, lymphoid thyroidosis, chronic non-specific thyroiditis, atrophic thyroiditis, primary myxoedema, autoimmune thyroiditis).
   - B. Focal lymphoid thyroiditis (autoimmune thyroiditis).
4. Riedel's thyroiditis (Riedel's struma, struma fibrosa, ligneous thyroiditis, chronic productive thyroiditis, invasive fibrous thyroiditis).

PURPOSE OF THE INVESTIGATION

The cytological diagnosis of non-malignant conditions of the thyroid has received relatively little attention and only few authors have stressed the value of the information obtainable from cytological examination of punctates in the treatment of various thyroid disorders.

The purposes of the present investigation were:

1. to describe the cytological picture of smears in acute suppurative, subacute, diffuse lymphoid and focal lymphoid thyroiditis,
2. to elucidate the cytological picture of diffuse lymphoid thyroiditis in patients of different ages,
3. to chart changes in the cytological picture of diffuse lymphoid thyroiditis in adults, as reflected in repeated biopsy specimens,
4. to compare the cytological and histological picture of diffuse lymphoid thyroiditis,
5. to assess the incidence of thyroid antibodies in adults with cytologically diagnosed diffuse lymphoid thyroiditis and to study the effect of treatment with thyroid hormone on the incidence of thyroid antibodies and to correlate the occurrence of thyroid antibodies with the cytological findings and
6. to report some clinical observations in adults with diffuse lymphoid thyroiditis, with special reference to the effect of treatment with thyroid hormone on size of the goitre.

CHAPTER II
METHODS

CYTOLOGICAL METHODS

Fine needle biopsy specimens for cytological examination

The conventional method with fine needle aspiration biopsy of the thyroid and light microscopical examination of the smears were used (52 198 and others) The smears were routinely stained with May - Grunwald Giemsa (MGG) stain and examined under low power lens and then under immersion lens in a Zeiss photomicroscope Kodachrome II A film was used for colour photographs and Agfa Isopan IFF film for black white photographs The size of the cells can be measured from the figures for magnification given in the photomicrographs As red blood cells are seen in most of the photomicrographs, assessment of the size of objects is also possible

All punctures were carried out by the author As a rule the thyroid was punctured at least twice to obtain material from different parts of the organ The needle was directed towards those parts of the gland that felt changed With the thin (23 gauge) needle used local anaesthesia was not required Only in the examination of children was a small volume of local anaesthetic injected and then with a 26-gauge needle

None of the 894 punctures of the thyroid was followed by complications The punctures were well tolerated by the patients Most of the patients had no discomfort at all after the puncture Some reported mild local pain the first few days after puncture when turning the head and when swallowing

Determination of the number of cells, the amount of colloid and the degree of polymorphism of follicular epithelium in diffuse lymphoid thyroiditis

The degree of polymorphism and the number of follicular epithelium and the amount of colloid were estimated from smears and classified according to the following scale 0 = none + = slight or scanty ++ = moderate +++ = marked or abundant The number of Askanazy cells was determined per 200 follicular epithelial cells When one and the same gland had been punctured more than once the above mentioned evaluations were based on a study of all the aspirates In those cases punctured twice the follicular epithelial cells per 1000 lymphoreticular cells and nucleated blood cells were also counted

The number of lymphoreticular cells was estimated as the number of cells per unit of surface in the most cellular smear. Since some lymphocytes and monocytes derive from an admixture of blood in the aspirate the number of cells was compared with that found in a smear of a peripheral blood sample obtained at the same time. The number of lymphoreticular cells was graded according to the following scale: o = none (lymphocytes and monocytes found derive from admixture of blood) + = scanty (single plasma cells and stem cells and slightly increased number of lymphocytes compared with that in peripheral blood smears) ++ = moderate +++ = abundant.

Since demonstration of lymphoretic

Table 1 *Eval at on of symbols (+) and cat ng number of lym horet cular cells by co nt ng the total number of mononucl at d cells from the blood and the lymphoret ular system per 1 mm² in the same sm ar. F g res in brackets ind cate the n mber of poly n l ated cells*

F le No	+	++	+++
7 65	51 (17)		
6(5 64	35 (11)		
361 64	8 (11)		
44 62	26 (1)		
Mean –	30 (10)		
39((4		36 (10)	
505 65		156 (4)	
24 (4		399 (0)	
371/62		74 (27)	
518 62		456 (4)	
5/(5		14 (21)	
M an		256 (11)	
602 (5			780 (7)
14((2			3208 (()
95 (2			1849 (19)
630 64			3910 (5)
590 64			4584 (5)
Me n			2866 (8)

+ = scanty + m d ra e – abundant

D fference betwe n the gr ups tested

f = 15 19 P < 0 1







F g 1 3 *Photom crographs of smears of thyro d asp rat s from pat ents w th d ffuse lymphoid thyro d t s. The reproduct ons illustrate the symbols used in the eval at on of the number of lymphor t lar cells. F g 1 (top) scanty (+) F le No 361 64 MGG 1 5 F g 2 (cent r) moderate ( ) F le No 396 64 MGC 175 F g 3 (bottom) ab ndant (++) F le No 146 62 MGG 1 5*

ular cells in an aspirate is a cardinal sign of diffuse lymphoid thyroiditis it was thought desirable to test the subjective connotation of the symbols +, ++ and +++ and to exemplify the picture designated by the symbols Table 1 shows the evaluation of the number of lymphoreticular cells in + symbols by counting the total number of lymphoreticular cells and mononucleated blood cells per unit of surface of the same smear The cases in the table were selected at random The mononucleated cells from the blood and lymphoreticular system were calculated for a surface of 1 mm² in the beginning and end of the smears (4 fields of vision with a magnification of ×250) It is clear from the table that the range of the values in the groups ++ and +++ was wide but they did not overlap The difference between the groups calculated with simple analysis of variance was significant** Fig 1—3 are microphotographs of cases with a cellular density close to average for the respective groups

Identification of Askanazy cells in MGG stained smears

Oxyphilic cells in the thyroid are known under different names such as Hurthle cells (103) Askanazy cells (6) and oncocytes (90) In thyroiditis they are generally called Askanazy cells and will be named so in this presentation The oxyphilic cells sometimes form adenomas (Hurthle cell adenoma Langhan s tumour)

Histologically Askanazy cells are defined as large cells with abundant oxyphilic or picrinophilic often fine granular cytoplasm The cells are identified best by their pink cytoplasm in haematoxylin-eosin stained sections (86) Descriptions and/or photographs of Hurthle cells in MGG-stained smears have been published by several workers in this field (28, 52, 125, 175 198) Since the number of published cases with an accompanying description of the histo-pathological diagnosis appears to be small, a supplementary investigation was considered legitimate

Fine needle biopsy specimens from subsequently histologically verified Hurthle cell adenoma were examined after being stained with May - Grunwald - Giemsa stain (Fig 39) or sometimes with haematoxylin eosin and haematoxylin-van Gieson The large epithelial cells of the Hurthle cell adenomas varied widely in size The nucleus was round or oval and contained 1—2 indistinct nucleoli The cytoplasm was usually sharply outlined In MGG stained smears the cytoplasm stained homogeneously slate coloured and exhibited a varying number of grey granules If the granules were numerous they often accumulated in one of the poles of the cytoplasm and then assumed a pale eosinophilic hue The granular texture of the cytoplasm was an invariable finding in the epithelial cells but it was sometimes so faint that it could only be seen under the immersion lens In smears stained with haematoxylin-eosin the cytoplasm stained pink (oxyphilic) and with haematoxylin van Gieson pale yellow (picrinophilic) The granules in the cytoplasm did not stain with any of the two last mentioned stains and

were discernible only as slight irregularities of the cytoplasm. The paravacuolar granules described by SÖDERSTRÖM (196) were not seen in MGG stained Hürthle cells. Small masses of colloid were sometimes seen.

Of the above characteristics, the size of the cells, the abundant cytoplasm and particularly the granular texture of the cytoplasm in MGG stained preparations are useful characteristics for identifying Askanazy cells.

Classification of lymphoreticular cells

Several systems have been used for classifying cells belonging to the lymphoreticular tissue in bone marrow and lymph nodes (58, 116, 138, 150, 165, 195 and others). This holds in particular for immature forms. Division into subgroups of stem cells can often result in very complicated systems. Even though it is possible to identify the different types of cells described in smears from lymphoid tissue, there will always be some cells that will not fit into any of the groups in the classification. Moreover, the nomenclature varies and makes it difficult to compare the results with those reported by other investigators. In the classification of the cells belonging to the lymphoid tissue in chronic thyroiditis attempts were made to use large, well defined groups, and after minor modifications the classification outlined by SÖDERSTRÖM (198) has proved useful for this purpose.

1 *Mature lymphocytoid cells* (Fig. 14, 56, 57)

To this group were assigned all small lymphocytoid cells resembling lymphocytes in the blood. The nuclei have a coarse chromatin network and as a rule no visible nucleoli. They lack a cytoplasm coating or are surrounded by a usually narrow area of basophilic cytoplasm.

The group "mature lymphocytoid cells" comprises lymphocytes from the blood and lymphoid tissue as well as various forms described by other authors as prolymphocytes (150), small, medium and large lymphocytes (58) and "Die kleinzelligen lymphoiden Reticulumzellen" (138, 165).

2 *Mature tissue bound reticulum cells* (Fig. 12, 57, 59)

These cells are readily identified: they have a round or usually an oval nucleus and a typical loose, netlike nuclear structure. One or two small, and often sharply defined, poorly staining nucleoli can be seen. The cytoplasm is abundant and stains pale blue. The outline of the cytoplasm is not distinct. The cytoplasm in some of the cells contains phagocytised material. The cells can therefore be divided into phagocytising and non-phagocytising tissue bound reticulum cells. Owing to the morphological similarities between the tissue bound reticulum cells and epithelioid cells and distinct transitional forms between the two types, in the differential count the epithelioid cells were assigned to the major group of tissue bound reticulum cells.

The phagocytising tissue bound reticulum cells correspond histo-pathologically in the lymph nodes to sinus-endothelial and other phagocytising reticuloendothelial cells.

3 *Lymphoglandular stem cells* (Fig 14 58, 59)

All immature lymphoreticular cells, which were characterised by a loose, fine "immature" nuclear structure and distinct and often large nucleoli were classified as stem cells No further subdivision was made in the differential count

4 *Plasma cells* (Fig 15, 57)

The mature plasma cells have the classical appearance known from haematology and histology Proplasma cells and immature plasma cells (57 116) were also assigned to this group

5 *Monocytoid cells*

The monocytoid cells comprise typical monocytes from the peripheral blood and monocyte like tissue cells They all have an oval or kidney-shaped nucleus poor in structural details and a slate grey cytoplasm The frequently irregularly stained cytoplasm is distinctly outlined and often contains eosinophilic granules and only little phagocytised material In the literature they are usually classified as histiocytes

6 *Large free phagocytes* (Fig 13)

These readily identified cells have abundant vacuolated well defined cytoplasm which often contains masses of phagocytised material The nucleus is round and is often masked by phagocytised material

Large free phagocytes differ in origin and in the thyroid they probably consist mainly of desquamated epithelial cells (198) In the literature they are also known as foamy phagocytes, cyst phagocytes macrophages or histiocytes In histological sections they are seen interstitially or intrafollicularly and are usually called macrophages

7 *Neutrophilic, eosinophilic and basophilic leukocytes*

These cells do not belong to the lymphoreticular system proper but were included in the original differential count Their appearance is well known from haematology No attempt was made to distinguish the basophilic leukocytes from tissue mast cells

8 *Mitoses* (Fig 14)

Mitoses of lymphoreticular cells were included in the differential count

Differential count of lymphoreticular cells in diffuse lymphoid thyroiditis

In each case studied with duplicate punctures 2000 lymphoreticular cells and nucleated blood cells were counted and distributed among the above mentioned groups In each aspirate then 1000 cells were counted Half of the cells was counted in the beginning and the other half in the end of the smear When several smears had been obtained from the same puncture the differential count was based on all smears

Seventy five cases in which two cytologically evaluable aspirates had been obtained were used for the differential count The cases excluded from the differential count belonged partly to the beginning of the investigation period when only one aspirate was obtained In addition those cases in which one of the aspirates could not be judged cytologically because of too small a yield or too large an admixture of blood were also excluded The admixture of blood

was probably due to the puncture needle passing through medium sized vessels

Most of the aspirates contained some admixture of blood, whose nucleated cells were included in the original differential count A differential count was made in a smear of blood from a finger tip collected at the same time as puncture The number of neutrophilic leukocytes was subtracted from the number found in the differential count of the thyroid aspirate, and according to the peripheral blood smear a corresponding number of lymphocytes, monocytes, basophilic and eosinophilic leukocytes was also subtracted The values were then given as a percentage This means that the number was not influenced by the admixture of peripheral blood and the differential count comprised only the lymphoreticular cells in the thyroid This correction presumes, of course, that all the neutrophilic leukocytes aspirated from the patients with lymphoid thyroiditis were included in the admixture of blood In none of the histological sections from patients with diffuse lymphoid thyroiditis could any extravascular polymorphonucleated leukocytes be demonstrated either

Sources of error and discussion of the method

The morphological variables in diffuse lymphoid thyroiditis have been based on the microscopical examination and differential count of epithelial cells and lymphoreticular cells in smears of fine needle aspirates These methods have several inherent sources of error, which are discussed below

Puncture and preparation of the smears

All punctures were performed and all smears were prepared by the present writer according to a standardised technique Differences in the results due to technical variation are thus negligible

The aspirated material may conceivably derive from the thyroid gland and at the same time from a nearby lymph node with the result that the appearance of the smear may simulate that of lymphoid thyroiditis From a practical point of view, however, this possibility may be ignored because in thyroiditis the follicular epithelium is changed and as a rule, the thyroid was punctured twice and the material was obtained from different parts of the gland

An admixture of blood in the aspirate is often unavoidable Correction was done in the way described above

Cell distribution in smears

The relative numbers of the different sorts of cells can vary from one smear to another from the same aspiration biopsy specimen because of an uneven distribution of the cells in the punctured part and unequal mixtures of the cells in the aspirated samples Cytological evaluation like the differential count of the Askanazy cells and lymphoreticular cells, was therefore based on a study of all smears The distribution of the cells may also differ from one part of a smear to another Large cells and groups of cells are often situated in the end of a smear It was therefore considered desirable to make a differential count of the cells in different parts of the smears In the middle of some of the smears however

the cells were so numerous and so crowded that they could not be identified with desirable certainty. For practical reasons counts of the lymphoreticular cells were therefore made in the beginning and the end of the smears.

Definition and identification of the cells

The cytological examination was based on the attenuated parts of the smears where the nuclei of the cells and the cytoplasm were most distinct. The follicular epithelial cells and the mature lymphoreticular cells were readily identified. Since all immature, lymphoreticular cells were taken together in a group of stem cells, classification of the immature lymphoreticular cells offered no difficulties either. In the beginning of the investigation an attempt was made to distinguish between large and small lymphocytes and between different types of stem cells. This division, however, appeared so uncertain that it was abandoned.

With the technique used the number of damaged cells was very small and, as a rule, no such cells were seen in the parts of the smears studied. A special group of damaged or unclassifiable cells was therefore considered unnecessary.

Source of error because of uneven distribution of cells in diffuse lymphoid thyroiditis

In an attempt to estimate the magnitude of this source of error in fine needle aspirates the cytological findings in the smears from two different parts of the same gland were compared in 12 randomly selected cases of diffuse lymphoid thyroiditis (Table 2). In order to avoid any subjective bias, the specimens were coded. The calculation also included all errors of the method except the statistically unavoidable error of the differential count, which is described later. No systematic error was found between aspirates I and II in the different components of thyroiditis. The standard error and the coefficient of variation of the different types of cells and the colloid are given in the table. In order to keep the error of the method for plasma cells and stem cells as small as possible the differential count was limited to cases where two punctures were made. The standard error and variation coefficient were thereby reduced by $\sqrt{2}^{-1}$ and for plasma cells they were $\pm$ 0.3 and 25.5 % respectively, and for the stem cells $\pm$ 0.5 and 29.4 % respectively and for the number of follicular epithelial cells/1000 lymphoreticular cells and nucleated blood cells $\pm$ 50.8 and 38.9 % respectively. Also for Askanazy cells the mean error was smaller than what is apparent from the table because also in most of these cases the cells were counted in two aspirates.

Statistically unavoidable error of the differential count of the cells

This error is entirely independent of other sources of error of the method and can be expressed in terms of the binomial distribution (146). The standard deviation of the binomial distribution of $\sqrt{\frac{p \cdot q}{n}}$ where p is the number of the counted specific cells, q the number of other counted cells and n the total

Table 2 *Total error of method (exc statistical error of cell count) of differential count of cells and determination of amount of colloid in duplicate thyroid punctures from 12 randomly selected cases of diffuse lymphoid thyroiditis*

File No	Askanazy cells in %			Colloid			No of follicular epithelial cells/1000 lymphoreticular cells			Plasma cells in %			Stem cells in %		
	Thyroid puncture			Thyroid puncture			Thyroid puncture			Thyroid puncture			Thyroid puncture		
	I	II	I—II (d)	I	II	I—II (d)	I	II	I—II (d)	I	II	I—II (d)	I	II	I—II (d)
197/62	[illegible]	[illegible]	0	−	+	0	43	105	−62	0.3	0.4	−0.1	0.6	1.6	−1.0
204/6[illegible]	0	0	[illegible]	+	−	0	37	47	−10	0.4	0.4	0	5.5	[illegible].7	+[illegible].8
362/6[illegible]	1.0	1.5	0.5	−	+	0	1.5	3	+1.2	0.6	0.4	+0.2	1.5	1.1	+0.4
366/6	22.0	26.5	−4.5	−	+	−1	[illegible]5	73	−48	1.7	1.1	−0.6	1.6	1.6	[illegible]
252/63	58.5	60.5	−2.0	−	−	0	38	36	+ 2	0.9	0.9	0	2.2	3.4	−1.2
90/65	0	0	0	−	+	0	70	59	+11	0.6	0.6	0	4.3	3.9	+0.4
[illegible]40/64	20.0	1.0	+19.0	+	−	[illegible]	2	25	−23	0.2	0.6	−0.4	1.1	1.6	−0.5
295/63	69.5	80.0	−10.5	+		+1	893	658	+[illegible]35	3.0	2.4	+0.6	1.4	0.5	+0.9
95/6[illegible]	76.5	89.0	−12.5	−	−	−1	34	78	−44	0.4	0.5	−0.1	1.4	0.6	+0.8
180/63	81.0	82.5	−1.5		−	−1	89	43	+46	1.0	1.4	−0.4	0.2	0.8	−0.6
42/63	7[illegible]5	58.0	−14.5	−	−	−1	290	99	+191	3.3	2.0	+1.3	0.8	0.9	−0.1
3[illegible]6/6[illegible]	42.0	41.0	+1.0	−	−	+1	172	91	+81	2.4	1.4	+1.0	1.4	1.6	−0.2
Mean	3[illegible].9	36.7		1.8	1.7		151.5	109.8		1.2	1.0		1.8	1.7	
$\sqrt{\frac{\Sigma d^2}{2n}}$		6.0			0.5			71.9			0.4			0.7	
Coefficient of variation		1[illegible].3 %			[illegible]8.6 %			55.0 %			36.1 %			41.6 %	

number of counted cells Since the types of cells counted are given as a percentage, $p + q = 100$ The statistically unavoidable error of the differential count of Askanazy cells from the 12 randomly selected cases is given in Table 3

As to other cytological findings such as multinucleated giant cells degenerative nuclear changes and paravacuolar granules the method had large inherent errors A large error of the method does not disqualify an investigation intended to demonstrate differences in the results between different age groups and at different times if in the evaluation the differences found exceed the error of the method In preliminary investigations however the differences in the above mentioned variables were so small compared with the error of the method that their evaluation was not included in this part of the investigation

Table 3 *Statistically unavoidable error of differential count (%) of epithelial cells and lymphoreticular cells in fine needle aspirates in 12 cases of diffuse lymphoid thyroiditis Same cases as in Table 2*

Statistical terms	Askanazy cells	Plasma cells	Stem cells
n	200	2000	2000
p	3[illegible].8 %	1.1 %	1.8 %
$\sqrt{\frac{p\ q}{n}}$	3.4	0.2	0.3
Coefficient of variation	9.[illegible] %	20.8 %	1[illegible].0 %

HISTOLOGICAL METHODS

Coarse needle biopsy specimens of the thyroid for histological examination were obtained after local anaesthesia with a Biegeleisen, Turner Warwick or modified Silverman needle. The specimens were removed by the surgeons Dr P Heimann and Dr L Risholm (for further description of the method, see 98). The specimens were examined histologically by Dr L B Schnurer. Woolner's (226) criteria for the histological diagnosis of lymphoid thyroiditis were used and are given in Chapter V (p 45).

STATISTICAL METHODS

The following abbreviations were used

n number of observations

d difference between two values

$\bar{x}, \bar{d}$ arithmetic means

s_x, s_d standard deviations

$s_{\bar{x}}, s_{\bar{d}}$ standard errors

t Student's t value when testing differences between two mean values

$y_x = bx + a$ estimate of the linear regression of y upon x;

r the correlation coefficient between two linearly related variables

t_b Student's t value when testing a regression coefficient b

F Fisher's distribution for quotients of χ^2 variables

The following conventions were employed to denote the level of significance: significant* ($0.01 < P < 0.05$), significant** ($0.001 < P < 0.01$) and significant*** ($P < 0.001$).

Statistical operations, such as calculation of method errors, coefficient of variations, correlation coefficient, analysis of difference between means, analysis of variances, multivariate linear regression analysis and χ^2 analysis were carried out by standard procedures (190). Yates' correction was used in χ^2 analysis when the expected frequencies were less than 1 (120).

CHAPTER III

CYTOLOGICAL PICTURE OF THYROID IN HEALTH AND IN ACUTE SUPPURATIVE, SUBACUTE, DIFFUSE LYMPHOID, FOCAL LYMPHOID AND RIEDEL'S THYROIDITIS

SURVEY OF LITERATURE ON THYROID CYTOLOGY

The practice of puncturing a tumour to find out the nature of its contents is old. As far back as 1879 CHUQUET described puncture in a case of peritoneal carcinomatosis (cit. BONNEAU et al., 20) GUTHRIE (80) is believed to have introduced puncture of lymph nodes and cytological examination of the aspirated material as a diagnostic method in 1921 In 1930 MARTIN & ELLIS (129) reported puncture of organs and gave detailed descriptions of the methods used at the Memorial Hospital in New York. Until 1933 they had performed as many as 2500 punctures including 45 punctures of the thyroid (193) Since then several reports of thyroid puncture for cytological diagnosis have been published either as part of a report of a tumour series (20 51 67 73 74, 191, 211) or of monographs on clinical cytology (1, 125, 194, 197 198) or as separate papers (5, 19, 27 28 52, 62, 79, 126 132 136, 141 142, 144, 149 152, 155, 175 187 196, 201 202)

Most of the above publications are descriptions of the cytological diagnosis on the basis of fine needle biopsy and are concerned with malignant conditions of the thyroid. The cytological diagnosis of thyroiditis was first mentioned in 1933 by STEWART (193), who felt that in the haematoxylin-eosin-stained smear "struma lymphomatosa is readily confused with the small cell carcinoma of the thyroid, even in sections Such being the case, it is unlikely that the two conditions can be diagnosed from smears The first case of subacute thyroiditis diagnosed cytologically was that reported by SODERSTRÖM in 1952 (196) The legend of one of the illustrations in a monograph on clinical cytology in 1954 by LOPES CARDOZO (125) gives a description of the cytological picture of struma lymphomatosa. Later other authors published some cases of thyroiditis (27 28 52, 62 126, 142, 144 149, 152, 175, 187, 198) The most exhaustive descriptions of Hashimoto's thyroiditis have been given by CERVINO et al. (27), NIKITINA (142) and SODERSTROM (198)

MATERIAL

The thyroiditis series formed a part of 894 patients in whom the thyroid was punctured to obtain cytological material

at some time between January 1962 and March 1965 The patients were subjected to thyroid puncture from various departments of Sahlgren s hospital and most of them were ambulant Puncture was done as a step in the investigation of thyroid functional disorders and/or of goitre In some patients the thyroid was not palpable at the time of puncture The youngest patient was 5 years old and the oldest 89 All persons were residents of Gothenburg

Normal thyroid Fine needle biopsy specimens were obtained from 8 euthyroid persons (4 men and 4 women), aged 25—58, without any history of thyroid disease and without palpable thyroid enlargement Some of the persons had some disease unrelated to the thyroid such as cervical rhizopathy, hypertension branchial cyst or psychoneurosis

Acute suppurative thyroiditis One case of acute suppurative thyroiditis in a previously healthy 48 year old man was included in the material After an infection of the upper respiratory tract he had fever and a tender goitre White blood count 10 000/mm³ Culture of a thyroid punctate gave growth of *a* streptococci

Subacute thyroiditis The series consisted of 20 cases of clinically diagnosed subacute thyroiditis All of the patients had pain in the region of the neck a tender goitre and a varying degree of general malaise The other most important clinical data results of laboratory studies and choice of treatment are given in Table 4 Treatment with prednisone had a prompt effect on the local signs and symptoms and fever At the review 6 months to 4 years later all of the patients were euthyroid and symptom free No goitre could be palpated except in 3 patients (Nos 6 8 and 17) who had a small firm swelling at the site of the previous inflammation

Diffuse lymphoid thyroiditis The series comprised 127 patients and was divided into two groups To the first group were assigned 37 patients with histologically verified diagnosis The material for the histological examination was obtained at partial thyroidectomy in 14 cases by coarse needle biopsy in 22 and by coarse needle biopsy and at partial thyroidectomy in 1

The other group consisted of 90 cases with only a cytologically verified diagnosis The cytological picture in this group agreed with that in the first group with a histologically verified diagnosis With a Biegeleisen- Turner Warwick- or Silverman needle attempts were made to obtain material for histological verification of the diagnosis in 19 cases In 12 of these, however no thyroid tissue was obtained In the remaining 7 cases the histological picture was possibly consistent with diffuse lymphoid thyroiditis but the material was too scanty to allow a firm diagnosis

Focal lymphoid thyroiditis Seventeen cases were selected retrospectively after histological examination of operative specimens of atoxic and toxic goitres They were classified as mild or severe focal thyroiditis according to the number of lymphoid foci per unit of surface (212 220) The mild group consisted of 5 cases with less than 10 foci and the severe group of 12 cases with at least 10

Table 4. *Material of subacute thyroiditis*
Clinical data laboratory tests and type of treatment.

Patient	Sex	Age	Fever	Sedimentation rate in mm in the acute phase	after recovery	PBI µg 100 ml	^{131}I uptake tests in % after 24 hours	Scintigram	Treatment
1 AD	F	57	—	54	8	8 [illegible]	1		Prednisone L-thyroxin
2 EN	F	47	—	1[illegible]0	15	6 0	1		L-thyroxin
3 SNK	F	48	—	110	[illegible]6	12 5	5		Prednisone L-thyroxin
4 PK	F	39	— —	80	14	8 6	0		Prednisone L-thyroxin
5 LG	M	42	— —	55	2	6 0	3		L-thyroxin
6 BJ	F	41	—	82	3	11 0	0		
7 RL	F	56	— —	126	3	8 6	0		Prednisone
8 SR	F	40	— —	92	7	10 0	1		Oxyphenbutazone
9 GG	F	4[illegible]	— —	73	9	10 6	1		Prednisone
10 FD	F	63	— — —	89	27	4 5	41	"Cold nodulus"	Penicillin Streptomycin
11 HE	F	48	— —	85	23	6 2	23	"Cold nodulus"	
12 IB	F	39	—	8	8		21	"Cold nodulus"	Prednisone Thiamazol
13 PO	F	47	—	4[illegible]	8	6 8	24	"Cold nodulus"	Phenylbutazone
14 ILA	F	47	— —	117	14				L-thyroxin
15 GM	F	37	[illegible]	48	4	10 8			L-thyroxin
16 JW	M	48	—	76	2				Prednisone
17 SW	F	[illegible]6	— —	48	7	5 3			L-thyroxin
18 GO	F	49	—	49	4				Prednisone L-thyroxin
19 DO	F	37	— — —	115	14				Prednisone
20 KB	F	22	— —	15	2				Prednisone

Normal values. PBI (= serum protein-bound iodine) — 4 8 µg 100 ml
^{131}I uptake after 24 hours 20 40 %
Fever — = mild — — = moderate — — — = marked

lymphoid foci cm² The number of lymphoid foci was determined in 6 fields of vision with a magnification of × 30

Focal lymphoid thyroiditis was diagnosed histologically when focal collections of lymphocytes and plasma cells in contact with follicular epithelium were seen (e g 212) Lymph follicles with germinal centres were invariably present. The occurrence of Askanazy cells was not considered necessary for a diagnosis of focal lymphoid thyroiditis but in all cases such cells were seen adjacent to one or more lymphoid foci As a rule there was also slight fibrosis

Thyroid puncture was performed some days before the operation in those patients who had received preoperative treatment with iodine, thyrostatics or thyroid hormones In the untreated group the interval between puncture and operation was within 5 months

Riedel's thyroiditis Only one case of Riedel's thyroiditis was available and included in the investigation The diagnosis was based on histological examination of material obtained at total thyroidectomy

RESULTS

Normal thyroid

Only every other puncture contained thyroid cells Follicular epithelium was usually found in clusters of 3—10 cells with a monomorphous picture (Fig 40) The nuclei were round and varied only slightly in size and stainability Nucleoli could not be discerned in the loose delicate chromatin network The cells formed a pseudosyncytium and no cellular borders were seen between the different epithelial cells or against the surrounding substance The faint grey-blue cytoplasm regularly contained small blue black granules within or adjacent small vacuoles and called paravacuolar granules by SODERSTROM (196) The number of paravacuolar granules varied from one group of follicular epithelial cells to another as well as from one case to another

Colloid was seen in all cases usually in the form of small blue irregularly outlined masses (Fig 40)

Acute suppurative thyroiditis

Acute phase The first puncture was performed in the febrile period when the gland was markedly enlarged and tender A pus like fluid was aspirated from the right lobe and the left The aspirate from the isthmus was slightly bloodstained

The microscopical picture was that of an acute suppurative process (Fig 41) The ground substance consisted of protein rich fluid containing necrotic material and numerous inflammatory cells Polymorphonucleated neutrophilic leukocytes constituted more than two thirds of the cells The rest of the inflammatory cells were mononucleated and contained an abundant, markedly vacuolated cytoplasm rich in phagocytised material Several of these macrophages were so-called large free phagocytes The smears also contained gram-positive cocci some of which were situated intracellularly (Fig 41)

Identifiable follicular epithelium occurred only in aspirates from the isthmus and showed pronounced degenerative changes Colloid was seen in the form of small masses

Convalescent phase (Fig 4 5) The patient was treated with antibiotics and puncture was repeated during the convalescent phase six weeks after the first puncture The needle was directed towards a residual grape-sized swelling at the site of the right thyroid lobe

Microscopical examination revealed a protein rich fluid chronic inflammatory cells (Fig 4) and cells from granulation tissue The neutrophilic leukocytes had been replaced by mature lymphocytes single plasma cells stem cells and multinucleated giant cells of foreign body type There were still numerous phagocytising mononucleated cells which together with fibroblasts and fibrocytes often formed small cellular clusters (Fig 5) Only a few colloid masses and a group of follicular epithelial cells with proliferative changes were seen

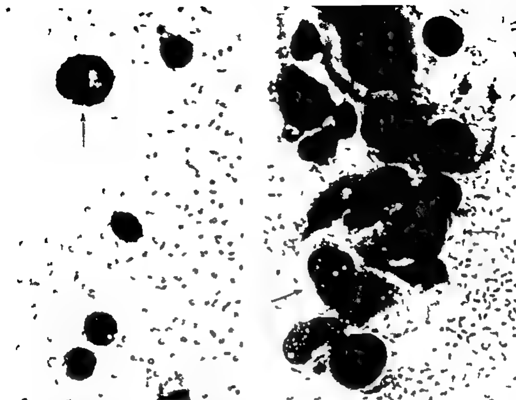


Fig 4–5 *Recovery phase of acute suppurative thyroiditis (File No 401/65 MGG N 2050) Fig 4 (left) Mature lymphocytoid cells and a plasma cell (arrow) scattered in a protein-rich fluid Fig 5 (right) Cluster of fibroblasts and macrophages (arrows) surrounded by protein rich fluid*

Subacute thyroiditis

General observations Owing to the frequently marked fibrosis in subacute thyroiditis it was often necessary to apply considerable force to introduce the fine biopsy needle into the goitre Patients with a very tender and hard goitre in contrast to those with other thyroid diseases, often found fine needle puncture painful

The aspirated material varied in amount and appearance In some instances it consisted only of a scanty protein-rich fluid containing no cells or colloid Other aspirates contained abundant epithelial and inflammatory cells with a widely varying admixture of blood

Follicular epithelium (Fig 42, 43, 44, 45, 48) occurred as solitary cells or clusters of cells without identifiable outlines The number of free epithelial cells increased with advancing degenerative epithelial changes Numerous paravacuolar granules degenerative and proliferative changes of follicular epithelium were seen in a varying amount depending on the stage of the subacute thyroiditis

The paravacuolar granules formed, large, blue-black granules and aggregates within or adjacent the often enlarged vacuoles in the cytoplasm (Fig 42, 43) They were invariably characteristic of the morphologically otherwise unchanged follicular epithelium The paravacuolar granules decreased in number and size or disappeared in the proliferating (Fig 45) and in the very degenerated (Fig 44) epithelium and appeared to be most numerous in the first stage of subacute thyroiditis

Degenerative changes of the epithelium varied strongly in degree and were seen in all cases except one in which the thyroid aspirate had been obtained late in the course of the disease The changes were most pronounced in aspirates obtained in the acute phase or from recently inflamed areas The degenerative changes were seen first in the cytoplasm which was swollen, faintly stained and cloudy (Fig 43 44) The cytoplasm sometimes showed diffusely outlined vacuoles and obviously phagocytised material Karyorrhexis was rarely seen and then only in cases with the most pronounced degenerative changes In some cases unclassifiable, intensely stained cells with pyknotic nuclei were seen

The number of proliferating follicular epithelial cells (Fig 45) varied inversely with the phase of subacute thyroiditis In 3 cases with severe degenerative changes no signs of follicular epithelial proliferation were seen

Inflammatory cells (Fig 6 46 47) were found in all cases They varied in amount from one aspirate to another Usually there was a moderate amount of inflammatory cells, often focal and situated near the epithelial cells Only one smear was crowded with inflammatory cells

The distribution of the inflammatory cells varied and consisted of a mixture of acute and chronic forms (Fig 46) Macrophages and lymphocytes were regularly seen and were often the dominant types of cells 'Macrophage is a blanket name for a heterogeneous group of phagocytising cells of varying appearance and origin (Fig 6 46 47) The lymphocytes were never the dominating type of cells as in diffuse lymphoid thyroiditis Neutrophilic leukocytes were seen in 14 of the 20 cases of subacute thyroiditis examined and could usually be readily distinguished from the leukocytes in the admixture of blood because they occurred in clusters (Fig 46) or were close to the follicular epithelium The neutrophilic leukocytes were usually less numerous than macrophages and lymphocytes Only in one aspirate numerous neutrophilic leukocytes were seen suggesting that a suppurative process had developed (Fig 6) No bacteria could however be demonstrated in direct preparations or on culture from this case and typical multinucleated giant cells were found in the aspirate from another part of the goitre As a rule, plasma cells were missing Neither the number of eosinophilic nor that of basophilic leukocytes was increased

Under low magnification the *multinucleated giant cells* (Fig 7, 48 49) were characteristic and readily identified They were sometimes missing in aspirates poor in cells but regularly occurred in one of





Fig. 6–7. Subacute thyroiditis. Fig. 6 (left). Numerous neutrophil leukocytes and macrophages (arrows) were an atypical finding in this case (Lab. No. 22[illegible] M.C.C. [illegible]1950). Fig. 7 (right). Multinucleated giant cell (at top) situated adjacent to a mass of colloid and probably [illegible] [illegible]. The nuclei are oval and eccentric. (Lab. No. 9[illegible] M.C.C. [illegible])

the aspirates from patients with subacute thyroiditis. They generally contained some 30 nuclei but sometimes up to 300 nuclei were counted in one giant cell. The nuclei were often situated on top of one another and occupied an eccentric position in the cell (Fig. 7). The nuclei were round or oval, had a loose chromatin network and 1 or 2 small, often faintly stained nucleoli surrounded by a homogenous, grey-blue, often well defined cytoplasm. Some of the multinucleated giant cells were of foreign body type and were sometimes situated adjacent to a mass of colloid (Fig. 7). Phagocytised nuclear remnants were sometimes seen. Morphologically, some of the cells resembled follicular epithelium and a few of the multinucleated giant cells contained paravacuolar granules (Fig. 49).

Scattered masses of *colloid* (Fig. 7) were found in 17 of the cases. The colloid was not so abundant as to form a ground substance in the smears.

Fibroblasts and *fibrocytes* distributed

irregularly in the smears were seen in more than half of the punctates

Diffuse lymphoid thyroiditis

Cytologically evaluable fine needle aspirates

The number of fine needle punctures performed in cases of diffuse lymphoid thyroiditis is given in Table 5. The punctures of different parts of the thyroid gland were performed at one and the same sitting. It is clear from the table that usually two punctures were performed.

Table 5. *Fine needle aspiration biopsy specimens obtained at first examination of patients with diffuse lymphoid thyroiditis*

Diagnosis	One puncture	Two punctures	More than two punctures	Total
Histologically verified diffuse lymphoid thyroiditis	14	20	3	37
Only cytologically diagnosed diffuse lymphoid thyroiditis	4	84	2	90
Total	18	104	5	127

The aspirate was accepted as sufficient for cytological examination if the smear was more than 1 cm² and did not contain too large an admixture of blood or cystic fluid. It was usually possible to judge with the naked eye whether sufficient material was obtained and as a rule also whether the punctate contained tissue cells. The material had a 'granular' appearance and small tissue fragments were often seen at the end of the smear.

Aspirates from 35 of the 37 histologically verified cases of diffuse lymphoid thyroiditis were accepted as satisfactory.

As is apparent from Table 6, in diffuse lymphoid thyroiditis it is more difficult to obtain sufficient material for cytological evaluation by fine needle puncture than in most other thyroid diseases.

Main cytological findings in fine needle aspirates and criteria for cytological diagnosis of diffuse lymphoid thyroiditis

Under low magnification the smears were seen to contain lymphoreticular cells and follicular epithelial cells scattered in a varying amount of blood. In exceptional cases the admixture of colloid was so massive that it constitutes a ground substance in the smears. Connective tissue cells and capillaries occurred only occasionally (Fig. 8).

The main cytological findings in

Table 6. *Cytologically evaluable material obtained at duplicate consecutive fine needle biopsies of patients with thyroid diseases. Figures in brackets represent number in per cent*

Cytological diagnosis	Number of patients in whom thyroid was punctured twice	Both aspirates evaluable
Diffuse lymphoid thyroiditis	102	76 (75)
Colloid goitre	102	83 (81)
Malignant or suspected malignant disease	77	71 (92)

Difference between groups diffuse lymphoid thyroiditis and colloid goitre: $\chi^2 = 1.40$, $P < 0.30$

Difference between groups diffuse lymphoid thyroiditis and malignant or suspected malignant disease: $\chi^2 = 9.36$, $P < 0.005$



Fig. 8. *Diffuse lymphoid thyroiditis. Row of endothelial cells from a dilated capillary and a cluster of fibroblasts and fibrocytes (arrows) surrounded by lymphoreticular cells (Fig. No. 433/6 MGG ×420)*

histologically verified diffuse lymphoid thyroiditis are given in Table 7 which also gives the frequency distribution of the cytological evaluation in + symbols.

Follicular epithelial cells occurred mainly in clusters in the end or along the edges of the smears. In all the cases there was a varying degree of cellular polymorphism. Microscopically, this polymorphism was characterised by variation in the size of the nuclei, an abnormally large mean diameter of nuclei and prominent nucleoli. When the polymorphism was more pronounced there were also variations of shape and stainability of the nuclei and size of the nucleoli. Askanazy cells and colloid were variable findings in aspirates from patients with diffuse lymphoid thyroiditis.

The picture was usually dominated by lymphoreticular cells consisting mostly of mature lymphocytoid cells. They were distributed over the entire smear.

The cytological findings in histologically verified diffuse lymphoid thyroiditis, when sufficient material for cytological examination is aspirated, are at the same time the criteria of diffuse lymphoid thyroiditis in smears and may be summarised as follows:

1. Follicular epithelial polymorphism
2. Moderate (+ +) or abundant (+ + +) number of lymphoreticular cells

Table 7 also gives the cytological findings in the group of diffuse lymphoid thyroiditis diagnosed only cytologically. In this group the diagnosis was based on the cytological picture of the fine needle aspirates and satisfied the criteria for a diagnosis of diffuse lymphoid thyroiditis. The frequency distribution of the amount of lymphoreticular cells and the degree of follicular epithelial polymorphism in histologically verified diffuse lymphoid thyroiditis did not differ significantly from that in cases diagnosed only cytologically. In the further analysis these two groups were pooled.

Detailed description of the cytological findings in different age groups

Follicular epithelium (Fig. 10, 11, 50, 51, 52, 53, 54, 55). The amount of aspirated

Table 7 *Frequency distribution of cytological evaluation of number of cells and degree of follicular epithelial polymorphism and amount of colloid in 37 patients with histologically verified and in 90 patients with only cytologically diagnosed diffuse lymphoid thyroiditis*

Cytological findings	Cytological evaluation							
	Histologically verified				Only cytologically diagnosed			
	■	+	++	+++	o	+	++	+++
Number of follicular epithelial cells		1	21	15		1	27	62
Degree of follicular epithelial polymorphism		1	16	20		17	29	44
Amount of colloid	11	22	1	3	9	72	3	6
Number of lymphoreticular cells		2*	13	22			25	65

* — Material insufficient for cytological diagnosis
o None + slight or scanty ++ — moderate +++ = marked or abundant
Difference between histologically and only cytologically diagnosed thyroiditis after pooling of groups + and ++ Follicular epithelial polymorphism $\chi^2 = o\ 28$ $P < o\ 60$ Lymphoreticular cells χ^2 1 98 $P < o\ 20$

follicular epithelium varied from one aspirate to another In those cases where two aspirates were obtained the number of follicular epithelial cells/1000 lymphoreticular cells and nucleated blood cells was determined and related to the patient s age The range of variation within the age groups varied widely No correlation was found in the ratio between the number of follicular cells and that of lymphoreticular cells with age ($r = 0\ 15$)

The follicular epithelium showed a variegated picture but no specific changes in diffuse lymphoid thyroiditis "Normal" follicular epithelium, proliferating epithelial changes and Askanazy cells were the three main types of epithelium

'*Normal*' follicular epithelium was seen above all, in young patients and as a rule had paravacuolar granules (Fig 10 50) *Proliferative* epithelial changes (Fig 51 53) were invariable findings and were a component of the previously described follicular epithelial polymorphism

The number of *Askanazy cells* per 200 follicular epithelial cells counted was correlated to the patient s age (Fig 9) The series consisted of all patients from whom duplicate aspirates were obtained Askanazy cells were rarely seen during childhood and puberty There was a significant*** correlation between the relative number of Askanazy cells and the patients ages

The cytological appearance of the Askanazy cells in MGG stained smears was described previously (page 11) In diffuse lymphoid thyroiditis the Askanazy cells were sometimes more polymorphic than in Hurthle cell adenoma and varied widely in shape size and stainability (Fig 10 52 53, 54 55)

Degenerative changes of follicular epithelium (Fig 54 55) were not common and occurred mainly in groups of

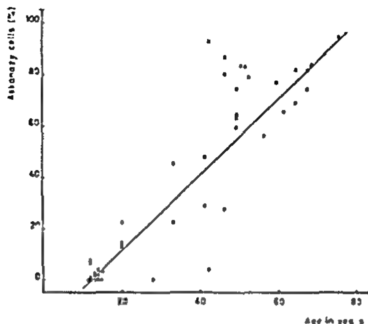


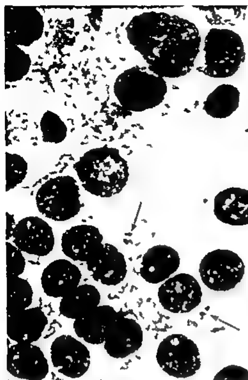
Fig. 9. Variation of relative number of Askanazy cells with age in diagnostic aspirates from [illegible] patients with diffuse lymphoid thyroiditis [illegible] 1.49 [illegible] 18 [illegible] 0.81 [illegible] 11.79 P [illegible] 0.001

Askanazy cells. They were seen mainly in the form of pyknosis and karyorrhexis (Fig. 55).

The variegated cytological picture of diffuse lymphoid thyroiditis sometimes included *multinucleated giant cells* (Fig. 11). The number of these cells often varied from one part of the gland to another and from one occasion to another. They contained 4–15 nuclei often lying on top of one another. The nuclei were round or oval and had a loose chromatin network. A grey-blue non-vacuolated cytoplasm surrounded the nuclei. Some of the cells resembled epithelioid cells, but in most cases follicular epithelium. They were sometimes reminiscent of multinucleated giant cells in subacute thyroiditis and [illegible]. Their cytoplasm contained no phagocytised material.

Colloid. The aspirate usually contained only scanty colloid and no colloid at all in 25 of 125 cases. In 9 cases there was abundant colloid and in 4 of them the colloid formed a ground substance in the smears. No significant difference was found in the amount of colloid between the 10–19 year age group and the 20–79 year age group ($\chi^2 = 1.91$, $P < 0.20$). The colloid was often seen as a small mass close to some follicular epithelial cells (Fig. 51).

Lymphoreticular cells (Figs. 1, 2, 3, 8, 11, 12, 13, 14, 15, 56, 57, 58, 59). As previously mentioned the lymphoreticular cells were usually predominant in the smears. The types of cells and their



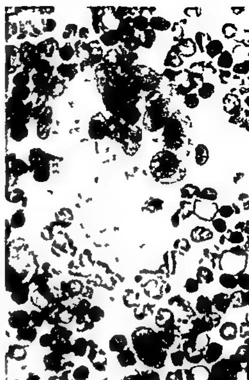


Fig 10—11 *Diff se lympho d thyroid tis Fig 10 (left) Normal foll cı lar epıthelı m (at bottom) with para ac olar gran les (arrows) and Askanazy cells (at top) Compare the polyn orpho s p ct ıre and the gran lat on ın the cytoplasm of the Askanazy cells with the monon orpho s pict ıre of the norn al epıthel m (File No 23/65 MGG × 1050) Fig 11 (r ght) Mult n cleated g ant cell of epıthel al orıg n and s ır ro nded by lymphoret cı lar cells (File No 1 9/62 MGG × 538)*

distribution in the different age groups, in those cases of lymphoid thyroiditis where aspirates were obtained from two different parts of the gland are given in Table 8 *Mature lymphocytoid cells* (Fig 12 14 56 57) constituted the dominating type (95 %) in all age groups *Mature tissue bound retıcı lum cells* (Fig 12 57 59) were invariably present and varied little in occurrence and phagocytising activity with the patients ages The number of these cells in the entire material was 1 2 % of the lymphoreticular cells

Monocytoid cells and *large free phagocytes* (Fig 13) were scanty No true tissue eosinophilia or basophilia was seen

The relative number of *stem cells* was significantly* higher in the 10—19 than in 20—79 year age group (Table 8) The stem cells were dominated by immature cells with scanty basophilic cytoplasm (Fig 14 58) (Soderstrom s NBC cell 198)

The relative number of *plasma cells* was lowest in the low age groups and increased successively after 40 years of

Table 8 *Differential count (%) of lymphoreticular cells of diffuse lymphoid thyroiditis in various age groups*

Age in years	10—19		20—39		40—59		60—79	
Number of patients	19		13		29		14	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Mature lymphocytoid cells	91.5—98.6	95.3	87.9—98.4	95.5	87.3—98.2	95.2	92.8—97.0	94.8
Mature tissue bound reticulum cells: with phagocytosis	0—1.3	0.3	0—1.1	0.3	0—1.0	0.3	0—1.0	0.4
Mature tissue bound reticulum cells: without phagocytosis	0—1.8	0.6	0.2—3.3	1.1	0.2—3.9	0.9	0.2—3.9	0.9
Mature tissue bound reticulum cells: total	0—1.8	0.9	0—3.3	1.4	0—3.9	1.2	0—3.9	1.3
Stem cells	0.5—6.6	3.1	0.5—8.9	2.3	0.3—6.5	2.3	0.3—3.4	1.7
Plasma cells	0.1—1.0	0.5	0.1—0.9	0.4	0.1—2.7	0.9	0.5—4.1	1.9
Monocytoid cells	0—0.6	0.1	0—1.4	0.4	0—2.4	0.3	0—0.7	0.2
Large free phagocytes	0—2.2	0.1					0—0.1	
Basophilic cells	0—0.2		0—0.3	0.1	0—0.4		0—0.3	0.1
Eosinophilic cells	0—0.1		0—0.2		0—0.3		0—0.3	0.1
Mitosis	0—0.1		0—0.1		0—0.1			

Difference between the age groups 10—19 and 20—79 years
Stem cells $|t| = 2.06$ $P < 0.05$
Plasma cells $|t| = 4.38$ $P < 0.001$

age (Table 8 and Fig 16) As for Askanazy cells there was a significant*** correlation between the relative number of plasma cells and the patients ages (Fig 16) Trivariate regression analysis with the percentage of Askanazy cells (y) as a function of the patients' ages (x_1) and the percentage of plasma cells (x_2) gave the following equation

$$y_x = 1.49\,x_1 - 0.17\,x_2 - 18.62$$
$$s_b = 0.15 \qquad 3.74$$
$$P < 0.001 \qquad < 0.98$$

This shows that the relative number of Askanazy cells was significantly*** correlated with the patients ages but not with the relative number of plasma cells

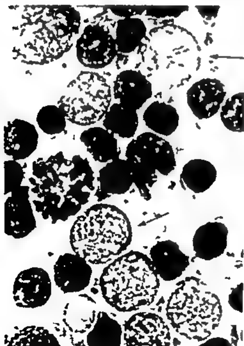
Plasma cells were invariably seen and often arranged in clusters adjacent mature tissue-bound reticulum cells (Fig 15)

Connective tissue cells In the presence of advanced fibrosis in diffuse lymphoid thyroiditis considerable resistance was

Fig 12—15 *Various forms of lymphoreticular cells in diffuse lymphoid thyroiditis Fig 12 (top left) Mature lymphocytoid cells mature non phagocytising (arrow a) and phagocytising (arrow b) tissue bound reticulum cells characterised by their oval nuclei loose chromatin network and lack of visible outline of cytoplasm (File No 108/65 MGG ×1050) Fig 13 (top right) Large free phagocytes (arrow a) with well defined outline of cytoplasm foamy appearance and abundant phagocytised material These cells may also be of epithelial origin Two lymphocytoid cells at arrows b (File No 98/62 MGG ×1050) Fig 14 (bottom left) Numerous mature lymphocytoid cells stem cells (arrows) and a mitosis (File No 413/62 MGG ×1050) Fig 15 (bottom right) Numerous plasma cells surrounding a mature tissue bound reticulum cell (arrow) This cell contains abundant phagocytised material (File No 101/63 MGG ×1050)*


b
a


a
b
b





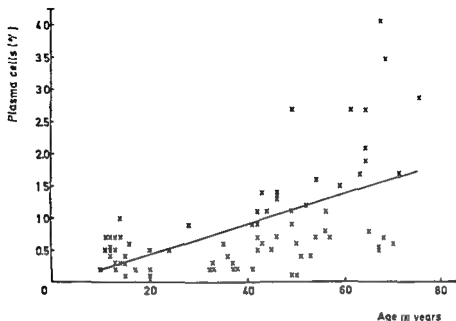


Fig 16 *Variation of relative number of plasma cells with age in duplicate thyroid aspirates from 75 patients with diffuse lymphoid thyroiditis* $y_x = 0.024x - 0.05$ $r = 0.57$ $t_0 = 5.89$ $P < 0.001$

offered to insertion of the biopsy needle Connective tissue cells were aspirated only exceptionally, and even then only as free fibroblasts or as fibroblast-fibrocytic foci

Focal lymphoid thyroiditis

The occurrence of lymphoreticular cells in cytological smears from patients with focal lymphoid thyroiditis is given in Table 9 In only 4 of all together 17 cases did the aspirates contain a slightly increased number of lymphocytes and single plasma cells, stem cells and tissue-bound reticulum cells The lympho reticular cells often occurred in clusters in the smears and in one case in only one of the two aspirates In no instance was the increase in the number of lymphoreticular cells so large that the cytological picture resembled that of diffuse lymphoid thyroiditis The major-

Table 9 *Lymphoreticular cells in fine needle biopsy specimens from patients with histologically diagnosed focal lymphoid thyroiditis* (— = *none* + = *scanty*)

Histological diagnosis	Lymphoreticular cells demonstrated in smear —	+	Total
Mild focal lymphoid thyroiditis (< 10 foci/cm)	4	1	5
Severe focal lymphoid thyroiditis (≧ 10 foci/cm)	9	3	12
Total	13	4	17





Fig 17–18 Riedel's thyroiditis (File No 137/65 MGG × 1,440) Fig 17 (left) Cluster of neutrophile leukocytes (arrow a) and a plasma cell (arrow b) Fig 18 (right) Three fibroblasts characterised by their spindle shaped nucleus and cytoplasm elongated towards poles Aggregates of thrombocytes (arrows)

in of the cases with histologically demonstrable focal lymphoid thyroiditis was thus not diagnosed cytologically

Riedel's thyroiditis

In the one case of Riedel's thyroiditis three punctures of a hard goitre produced only a scanty yield Microscopically the smears contained inflammatory cells connective tissue cells and a few follicular epithelial cells Mature lymphocytoid cells constituted half or two thirds of the inflammatory cells Neutrophilic leukocytes were the next commonest type They were often seen in clusters (Fig 17) Single plasma cells and macrophages completed the inflammatory cellular picture One of the aspirates contained some multinucleated giant cells Fibroblasts and fibrocytes (Fig 18) constituted about 10 % of the nucleated cells They occurred partly in densely crowded clusters Only a few groups of follicular epithelial cells were seen Their nuclei were crowded sometimes in layers The cytoplasm contained black blue granules which probably consisted of paravacuolar granules No Askanazy cells were seen in the aspirated material

DISCUSSION

Normal thyroid Knowledge of the normal cytological appearance of the thyroid is necessary for recognising any change

of the follicular epithelium in thyroiditis Therefore, the cytological picture of the thyroid was studied in some euthyroid persons without known thyroid disease The normal histological picture of the thyroid varies from country to country and district to district owing, among other things, to exogenous factors (88) SODERSTROM (198) prefers to speak of the standard rather than the normal cytological picture The material presented here is not intended to give a complete picture of thyroid cytology and its variation with age and geographical region, but only to describe the appearance of the follicular epithelium and the colloid found in some cases which may be regarded as normal in the district under consideration, *i e* Gothenburg

Acute suppurative thyroiditis Acute purulent thyroiditis is believed to be rare (92 216) As in the case reported here, it may appear as a complication of an infection of the upper airways in an otherwise healthy person (92) KOHLER et al (111) reported a case in which the clinical features and laboratory studies suggested typical subacute thyroiditis, but where intracellular bacteria were found on histological examination of a needle biopsy specimen The preliminary clinical diagnosis in the present case was *subacute thyroiditis or reticulum cell* sarcoma There is reason to believe that a more frequent use of needle biopsy for histological or cytological examination would result in a firmer differential diagnosis of acute thyroid diseases

The cytological picture of acute suppurative thyroiditis can be distinguished from that of acute inflammatory conditions in other organs only by the demonstration of colloid or follicular epithelium This was missing in two of the three punctates, which may be due to the size of the suppurative inflammation or overrepresentation of the readily aspirated necrotic and inflammatory material in the fine needle biopsy specimen. Confluent microabscesses may occur in subacute thyroiditis (93, 227) The occurrence of a large number of acute inflammatory cells in smears is thus not by itself sufficient to warrant a cytological diagnosis of acute suppurative thyroiditis As in the present case demonstration of intracellular bacteria is necessary for the cytological diagnosis

Once the acute stage of the suppurative thyroiditis had passed, repeated puncture showed a cytological picture of a reparative inflammatory process The process shows no characteristic features and in this stage it cannot be distinguished from the reparative stage of subacute thyroiditis

Subacute thyroiditis It is believed that in typical cases subacute thyroiditis can be diagnosed on clinical grounds alone (183) A fairly acute onset with neck pain, systemic symptoms, fever elevated E S R and a firm, tender goitre are characteristic features A therapeutic *response to prednisone corroborates the* diagnosis The patient makes a complete recovery, sometimes after a few recurrences Laboratory tests other than E S R , protein-bound iodine and determination of uptake of radioactive iodine are of little value (200) An increased amount of protein-bound iodine and decreased uptake of ^{131}I argues for

subacute thyroiditis Sometimes the uptake of ^{131}I may be normal but the scintigram shows a decreased uptake over the inflamed area (227)

The 20 cases in the present material may be regarded as clinically definite cases of subacute thyroiditis

In atypical cases of subacute thyroiditis biopsy is useful (186) Abortive cases with only mild local symptoms often remain concealed (36, 210) Patients with a hard and only slightly tender goitre are sometimes operated upon because of suspected cancer (210, 227) On the other hand a subacute thyroiditis can be simulated by a lymphadenoid goitre (45) acute suppurative thyroiditis (111) and bleeding in a goitre (93) Even if thyroid function studies and further observation reveal the nature of the disease, a simple and rapid method for confirming the diagnosis of subacute thyroiditis may sometimes be called for in the initial stage of the disease

Subacute thyroiditis often starts in one part of a lobe and then spreads to the other parts (creeping thyroiditis) This course of the disease explains the variegated picture of cytological smears prepared from fine needle aspirates The degenerative and proliferative changes of the epithelium like the inflammatory cells vary not only from case to case but also in aspirates from different parts of the same gland But constant characteristics are multinucleated giant cells and numerous paravacuolar granules in the otherwise morphologically normal follicular epithelium

Multinucleated giant cells are seen in many thyroid diseases but in subacute thyroiditis they are such a typical finding that they occur in several synonyms of subacute thyroiditis They are seen in both histological and cytological specimens, often with ingested colloid They are believed to be of both mesenchymal and epithelial origin (123) The latter origin is corroborated in the present material by their morphological similarity to follicular epithelium and the finding of paravacuolar granules in the cytoplasm in some giant cells (Fig 49)

Paravacuolar granules were first described by SODERSTROM (196) and are often seen in the normal thyroid in colloid goitre, subacute thyroiditis and thyrotoxicosis (198) SODERSTROM (196) thought them to be associated with the secretory function of the cells and to be located at the site of the Golgi apparatus In a radioautographic examination of thyroid smears from patients who had received an injection of ^{131}I SCHMIDT et al (176) found a blackening in the form of granules in the cytoplasm of the epithelial cells, but they said nothing about the position of the granules in relation to the paravacuolar granules The paravacuolar granules have a pale yellow autofluorescence when seen in ultraviolet light (153) They are abundant in the first phase of subacute thyroiditis, when regressive cellular changes occur and clinical signs of thyrotoxicosis may be seen It is not known to which ultra structure the paravacuolar granules correspond In electron microscopical studies HEIMANN (96) showed that dense bodies supposed to be lysosomes occur in normal human thyroid and in great numbers in human toxic goitre The

of the follicular epithelium in thyroiditis Therefore, the cytological picture of the thyroid was studied in some euthyroid persons without known thyroid disease The normal histological picture of the thyroid varies from country to country and district to district owing, among other things, to exogenous factors (88) SODERSTROM (198) prefers to speak of the standard rather than the normal cytological picture The material presented here is not intended to give a complete picture of thyroid cytology and its variation with age, and geographical region but only to describe the appearance of the follicular epithelium and the colloid found in some cases which may be regarded as normal in the district under consideration *i e* Gothenburg

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about one third of the cytological smears from patients with diffuse lymphoid thyroiditis and are sometimes prominent Histologically the multinucleated giant cells probably correspond to the proliferating intrafollicular cells which are sometimes seen to fill the follicles completely and often to be surrounded by well defined fibrosis (124) Some multinucleated giant cells are considered to be of mesenchymal origin (29) They are believed to be a common histological finding in a certain phase of lymphoid thyroiditis (124) An abundance of these granuloma like changes induced SODERSTROM (198) to distinguish a granuloma type of subacute thyroiditis which morphologically and clinically resembles Hashimoto s thyroiditis In the light of published histological examinations the varying occurrence of multinucleated giant cells in fine needle aspirates from different parts of the gland and at different times as well as the clinical course in the present material, there appears to be no reason to regard cases with numerous multinucleated giant cells as a special group of thyroiditis

The lymphoreticular cells are found in the thyroid diffusely scattered or arranged in lymph follicles In their germinal centre the latter contain immature cells which in the smears correspond to stem cells (116) Stem cells as a rule, constitute less than 8 9 % of the number of lymphocytes in the punctate (Table 8) This together with the bore and length of the puncture needle excludes the possibility that the aspirated material should derive exclusively from one or more germinal centres

The histological picture of lymphoid thyroiditis in children differs from that in adults It is widely agreed that fibrosis is less marked in children than in adults (33 83, 173) According to SAXENA & CRAWFORD (173) an eosinophilic degeneration of epithelium is common in children On the other hand, CLAYTON & JOHNSON (33) and HAHN JR et al (83) claim that oxyphilic epithelium in children is either missing or very scanty The present results corroborate the opinion of the last mentioned authors In addition, the relative number of Askanazy cells increases in the entire thyroiditis series with increasing age of the patient which according to LENNOX (117) also holds for normal and thyrotoxic thyroid gland with lymphoid hyperplasia ה focal lymphoid thyroiditis

Establishment of the diagnosis of diffuse lymphoid thyroiditis does not require a differential count of the lymphoreticular cells Such a count however reveals further differences in the picture of the thyroid between children and adults The relative number of stem cells is significantly* higher in children and adolescents than in adults HAHN JR et al (83) observed a prominent formation of germinal follicles in histological sections from 27 children with diffuse lymphoid thyroiditis The increased number of stem cells in cytological smears in the lower age groups may thus be a manifestation of a larger number of germinal centers or it may be a component of a more lively cell newformation during the years of growth

The percentage of plasma cells increases significantly *** with the patients' ages This also holds for plasma cells in bone marrow aspirates in different ages (165, 177) The increase in the relative number of the plasma cells with age in diffuse lymphoid thyroiditis may thus be only a part of a general increase in the number of plasma cells with age

A significant correlation was found between the three variables relative number of Askanazy cells, relative number of plasma cells and the patients' ages Since plasma cells can form antibodies (*e g* 57, 165) reacting with thyroid cells (102 157) the data obtained were studied with a multiple correlation analysis It was found that the relative number of Askanazy cells was significantly *** correlated with the patient s age but not with the number of plasma cells

In experimental studies on rats WILLIAMS and NOSSAL (221) and WILLIAMS (219) showed that the formation of 7S γ globulins was correlated in time with the appearance of medullary dendritic macrophages in lymph nodes and in the spleen It has therefore been suggested that the presence of macrophages is essential for the formation of 7S γ-globulins (145) to which the main part of thyroid autoantibodies belongs (204) In the present material mature tissue-bound reticulum cells (macrophages) were invariably seen sometimes surrounded by plasma cells (Fig 15) in a manner similar to that described by SODERSTROM (198) in a spleen aspirate from a case of rheumatoid arthritis and to the erythroblast foci observed in bone marrow by different authors (see 17)

The diagnosis of diffuse lymphoid thyroiditis is easy and offers hardly any differential diagnostic problems provided the aspirate is rich in cells It may, however, sometimes be difficult to distinguish from focal lymphoid thyroiditis if the aspirate contains a heavy admixture of blood because then the increase in the number of lymphocytes is less evident In such cases aspiration biopsy should be repeated to obtain a more representative yield In addition, in intermediate cases it may sometimes be difficult to decide whether the changes should be interpreted as focal or diffuse lymphoid thyroiditis Differentiation from subacute thyroiditis is easy On the other hand, in the presence of numerous epithelioid cells and multinucleated giant cells the picture may resemble that of sarcoidosis As pointed out by SODERSTROM (198), in such cases generalised sarcoidosis must be excluded The cytological diagnosis in coexisting malignant thyroid processes and thyroiditis is important and sometimes difficult It is discussed in Chapter V

The histo-pathological similarity between diffuse lymphoid thyroiditis and human toxic goitre has long been known (see 75) In most cases it is a question of a focal lymphoid thyroiditis but also pictures with certain parts of the gland indistinguishable from classic Hashimoto's disease have been described (23 77, 119) The relationship between thyrotoxicosis and autoimmune thyroiditis has been discussed (*e g* 48, 140) as have the antibody properties of long acting thyroid stimulator (13, 135) A positive

correlation has been found between the amount of lymphoid tissue in thyrotoxic glands and the frequency of myxoedema after subtotal thyroidectomy (77, 119, 218)

In patients with symptoms of thyrotoxicosis the histological picture is usually typical in a diffuse goitre but not in nodular goitre (for references see 99) Also the cytological picture of smears from thyrotoxic glands varies (141 198) Demonstration of thyroid hyperfunction in patients with coexisting lymphoid hyperplasia of the gland is thus not a cytological but a clinical problem The occurrence of lymphoreticular cells in aspirates of toxic goitre however indicates a risk of hypothyroidism after subtotal thyroidectomy and probably also after treatment with ^{131}I

Focal lymphoid thyroiditis Long term treatment with iodide may cause lymphoid infiltration of the thyroid in human beings (21) The thyroid changes characteristic of struma lymphomatosa are produced in rats by long term administration of thiouracil (32) In animal experiments it has been shown that lymphoid tissue in the body can also be influenced by administration of thyroxin (54 81, 127) The material of focal lymphoid thyroiditis presented therefore consists only of cases in which aspirates were obtained a day or so before thyroidectomy or which had not been treated with thyrostatics iodide or thyroid preparation

Focal lymphoid hyperplasia is a common histological finding in thyroid glands It is seen *inter alia* in thyrotoxicosis atoxic goitre and malignant thyroid processes At autopsy of 770 subjects in whom no signs of thyroid disease had been noted *intra vitam* and in whom the thyroid gland were of normal gross appearance WILLIAMS & DONIACH (220) found oxyphilic epithelial changes (Askanazy cells) and more than 10 lymphoid foci per cm² in 22 % of adult females and in 6 % of adult males Similar results have been reported by other authors (10 31, 76, 139 181, 212)

It is clear from Table 9 that focal lymphoid thyroiditis is reflected in the smears in only one fourth of the cases and then by only a few lymphoreticular cells Owing to the focal nature of the thyroiditis it is possible that only one of the aspirates contains lymphoreticular cells

A slight increase in the number of lymphoreticular cells is also sometimes seen in regressive thyroid changes which however, are recognised by the presence of iron pigment in the macrophages and membrane remnants from destroyed erythrocytes

Riedel s thyroiditis Only one case of the rare type of Riedel s thyroiditis was available for investigation The resistance offered to the needle as well as the occurrence of fibroblasts and fibrocytes in the smears were markedly increased but this did not distinguish Riedel s thyroiditis from the fibrous variant of diffuse lymphoid thyroiditis It differed from the latter condition in that Askanazy cells were missing and that the smear showed small neutrophilic leukocyte foci It should thus also be possible to diagnose Riedel s thyroiditis by fine needle biopsy

CHAPTER IV

REPEATED FINE NEEDLE ASPIRATION BIOPSY IN ADULTS WITH LYMPHOID THYROIDITIS

Autopsy studies have shown that the frequency of lymphoid thyroiditis, both focal and diffuse, increases with age (31, 76, 130, 139, 181, 212 220) It is believed that only a small proportion of the cases of focal lymphoid thyroiditis progress to the diffuse form (212, 220) The further natural course of diffuse lymphoid thyroiditis, once it has been established, is obscure Its response to treatment with thyroid hormone is not properly understood either Clinical observations suggest that the originally euthyroid patient may become hypothyroid and that the goitre may decrease in size and even disappear in association with such treatment (71, 134)

The course of lymphoid thyroiditis has been the subject of only few histological investigations In 1961 VICKERY and HAMLIN JR (209) gathered 21 cases from the literature, to which they added 16 personal cases from which biopsy specimens had been obtained on 2 or more occasions They found no histological change in the interim in 9 cases, a slight to mild increase in the amount of fibrosis in 4 and increase in the amount of lymphoid tissue in 3 and a decrease in 1 Parenchymal atrophy including increasing follicular cell oxyphilia was noted in 6 cases

DONIACH & ROITT (48) and SKILLERN (183) stressed the desirability of histological follow-up of lymphoid thyroiditis

METHODS

Fine needle biopsy specimens of the thyroid were obtained on 2 occasions from each patient The interval between the first and second puncture was 4—44 months (mean 20 months) The cytological criteria of diffuse lymphoid thyroiditis used were those described previously (page 26) Focal lymphoid thyroiditis was said to be present when the number of lymphoreticular cells was increased in only one of the aspirates or when the lymphoreticular cells were slightly increased in number and then often occurred in scattered clusters

In the group "diffuse lymphoid thyroiditis" the ratio between the number of follicular epithelial cells and lymphoreticular cells was calculated Only cases with 2 cytologically evaluable aspirates obtained on the first occasion were used for this analysis The ratio between the number of follicular epithelial cells per 1000 lymphoreticular cells and nucleated

Table 10 *Comparison between the cytological diagnosis on the basis of aspirates obtained on two occasions with an interval of 4–44 months in diffuse and focal lymphoid thyroiditis*

Cytological diagnosis on basis on material obtained on first occasion	Cytological diagnosis on basis on material obtained on the second occasion					Total
	Diffuse lymphoid thyroiditis	Focal lymphoid thyroiditis	Colloid goitre	Scanty material with appearance compatible with diffuse lymphoid thyroiditis	No thyroid cells	
Diffuse lymphoid thyroiditis	69	1		5	3	78
Focal lymphoid thyroiditis	3	6	2		1	12

blood cells was calculated in each case with two aspirates (x_1 and x_2). The standard error (s) of a single observation was calculated from the formula $\sqrt{\frac{\sum d}{2n}}$ where $d = x_1 - x_2$. Then $\bar{x} \pm 1.96\ s$ was determined.

Further details of the method are given in Chapter II.

The other group consisted of 16 patients in whom fine needle aspiration biopsy had given a diagnosis of focal lymphoid thyroiditis. Fine needle aspiration biopsy specimens were obtained on a later occasion in 12 (the remaining 4 could not be traced for the review). Ten of the 12 patients had in the meantime been treated with l-thyroxin 0.1–0.2 mg/day and 2 had received no treatment.

MATERIAL

The material consisted of two groups. The first comprised 78 of the 89 adults with diffuse lymphoid thyroiditis diagnosed by fine needle aspiration biopsy. Of the 11 patients not included, the thyroid was not palpable in 7 and was therefore not punctured, 2 refused re-puncture, 1 could not be traced for the review and 1 had died from an intercurrent disease. Of the 78 patients 61 had in the meantime been treated with thyroid hormone, usually l-thyroxin 0.1–0.3 mg/day. *Two had been treated* with l-thyroxin and thiamazol. Fifteen patients had received no treatment.

RESULTS

The cytological diagnoses in the repeated biopsies in the 2 groups are summarised in Table 10. In the patients treated with thyroid preparations it proved more difficult to obtain sufficient material for cytological examination on the second occasion than on the first. Thus in 8 of the 78 cases of diffuse lymphoid thyroiditis puncture on the second occasion produced but little or no material. In those cases where sufficient material was obtained from patients with diffuse lymphoid thyroiditis agreement in diagnosis was found between the punctures obtained on the two

occasions except in 1 case with the diagnosis of focal lymphoid thyroiditis in the repeated biopsy specimen (Table 10)

Among the 12 patients with focal lymphoid thyroiditis the diagnosis was the same in only half of the cases on the second occasion (Table 10) Diffuse lymphoid thyroiditis was diagnosed in 3 patients and colloid goitre in 2 Thyroid cells were missing in the aspirates from 1 patient

The cytological picture in the diffuse lymphoid thyroiditis group was analysed further The number of follicular epithelial cells and lymphoreticular cells in the aspirates from the first occasion and the second were counted for any change in the ratio between them (Table 11) Of the 51 patients the number of follicular epithelial cells per 1000 lymphoreticular cells and nucleated blood cells was larger on the second occasion than on the first in 10 and smaller in 1 The probability (P) of this difference being due to chance was 0 006 All of these cases with changed ratio belonged to the group treated with thyroid hormone The difference in this respect between the treated and the untreated group was significant*

In 48 randomly selected cases of diffuse lymphoid thyroiditis the percentage of Askanazy cells per 200 follicular epithelial cells in aspirates obtained on the first occasion was compared with the corresponding value found on the second occasion Of the 48 patients 37 had been treated with thyroid hormone (Fig 19) A significant*** increase in the relative number of Askanazy cells was found in the repeated biopsies No difference was found in this respect between the two groups, *i e* treated and untreated with thyroid hormone

As in the samples obtained on the first occasion the specimens contained a small amount of colloid which had further decreased in amount However, the difference in the amount of colloid between the first and second occasion was not significant ($|t| = 1\ 45$, $P < 0\ 20$) Neither was any significant difference found between cases treated with thyroid hormone and untreated cases ($|t| = 1\ 10$ $P < 0\ 30$)

Table 11 *Change in ratio between follicular epithelial cells and lymphoreticular cells in fine needle aspiration biopsy specimens from the second occasion compared with the first in cases of diffuse lymphoid thyroiditis treated with thyroid hormone and not treated with the hormone The groups increased and decreased comprised those cases in which the number of follicular epithelial cells per 1000 lymphoreticular cells and nucleated blood cells on the second occasion fell outside the range of $\bar{x} \pm 1\ 96\ \varepsilon$ where $\bar{x}$ = mean of observations (x_1 and x_2) in duplicate aspirates on the first occasion*

$$\varepsilon = \sqrt{\frac{\sum d^2}{2n}} = 94\ 2 \quad \text{where } d = x_1 - x_2$$

Treatment	Number of follicular epithelial cells per 1000 lymphoreticular cells and nucleated blood cells on the second occasion compared with those on first occasion			
	Increased	Unchanged	Decreased	Total
Thyroid hormone	10	27	1	38
No thyroid hormone	0	13	0	13
Total	10	40	1	51

Difference between the groups treated with thyroid hormone and those not treated after pooling of groups unchanged and decreased $\chi^2 = 4\ 26$ P [illegible] $0\ 05$

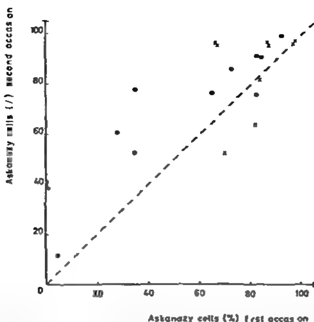


Fig 19 *Percentage of Askanazy cells of 100 epithelial cells counted on first and second occasions in 48 randomly selected cases of diffuse lymphoid thyroiditis in adults. Broken line drawn at 45°. x = treated with thyroid hormone. • = not treated with thyroid hormone. Difference in the percentage of Askanazy cells between the first and second occasions* $|t| = \frac{d}{s_d} = 4.73$ $P < 0.001$. *Difference between the groups x and •* $|t| = 1.24$ $P < 0.30$

DISCUSSION

It is well known that the histological picture of the thyroid can vary from one part of the gland to the other in patients with lymphoid thyroiditis. Therefore neither histological nor cytological examination of puncture biopsy specimens seem to be ideal methods for following the changes in morphology of diffuse lymphoid thyroiditis. In chapter II however it was shown that the agreement between the cytological findings in two needle aspiration biopsy specimens taken at the same time from different parts of the goitre was good concerning the incidence of Askanazy cells, stem cells and plasma cells and the amount of colloid. In the following chapter it will be shown that this also holds for the comparison between the cytological and histological picture in operated cases of diffuse lymphoid thyroiditis with the exception of fibrosis which cannot be demonstrated in cytological smears. Cytological examination of biopsy specimens may thus be used for studying the course of diffuse lymphoid thyroiditis.

The cytological diagnosis of the specimens obtained on the two occasions showed good agreement except in half of the cases of focal lymphoid thyroiditis. The discordance in these cases was

probably due to differences in various parts of the gland from which the material had been obtained on the two occasions rather than to a true change in the morphological picture of the glands

An increase in the ratio between the number of follicular epithelial cells and lymphoreticular cells was more common than a decrease in samples obtained on the second occasion The difference was significant** All of these cases had been treated with thyroid hormone and in all patients except one the goitre had decreased Treatment with thyroid hormone inhibits the production of the thyroid stimulating hormone (TSH) and thereby suppresses the stimulating effect of TSH on the follicular epithelium (see 42, 158) This together with a simultaneous regression of the enlargement argues against the possibility of the number of follicular epithelial cells increasing during treatment with thyroid hormone and being the cause of the increased ratio between the number of follicular epithelial cells and lymphoreticular cells Instead the increased ratio seems to be due to a decrease in the number of lymphoreticular cells Reduction of the lymphoid tissue in thyroiditis is thus probably the most important cause of the regression of goitre during treatment with thyroid hormone This would fit in well with the observation that the severe goitre in patients with diffuse lymphoid thyroiditis is due mainly to an increase of lymphoid tissue (119)

The amount of colloid in diffuse lymphoid thyroiditis was small and did not vary significantly in samples obtained on the first and on the second occasion and can therefore presumably have contributed little to the change of the goitre following treatment with thyroid hormone The role played by fibrosis in the decrease of the thyroid gland cannot be judged from fine needle biopsy specimens but it is probably of only subordinate importance (209)

In the previous chapter it was shown that the relative number of Askanazy cells in diffuse lymphoid thyroiditis increases with age and that the correlation is significant*** The relative number of Askanazy cells in samples obtained on the second occasion is accordingly significantly*** larger than on the first This suggests a continuous conversion of epithelial cells to Askanazy cells Since the onset of diffuse lymphoid thyroiditis cannot be dated with any degree of certainty it is not possible to say whether the increase in the relative number of Askanazy cells is related to the duration of thyroiditis or to the patient's age The former alternative appears more likely, especially as diffuse lymphoid thyroiditis is common in adolescence (113, 144, 173)

Treatment with thyroid hormone had no demonstrable effect on the increase of the relative number of Askanazy cells in the repeated biopsies Since the absolute number of such cells cannot be determined from examination of smears and since treatment with thyroid hormone can, by a feedback mechanism, affect the regeneration of follicular epithelium (42, 158) it is, however, not possible to draw any conclusion on the effect of treatment with thyroid hormone on the conversion of epithelial cells to Askanazy cells

CHAPTER V

COMPARISON BETWEEN CYTOLOGICAL AND HISTOLOGICAL FINDINGS IN DIFFUSE LYMPHOID THYROIDITIS AND IN LYMPHOID THYROIDITIS WITH COEXISTING MALIGNANT THYROID DISEASE

By

P. Sigvard Persson and Lars Bertil Schnürer

It is well known from histological investigations that in diffuse lymphoid thyroiditis the microscopical picture can vary from one part of the thyroid to the other. Yet histological examination of needle biopsy specimens of the gland is regarded as a reliable method for diagnosing thyroiditis (12, 37, 89, 95, 98, 134, 213).

Malignant thyroid disease is more common in the presence than in the absence of lymphoid thyroiditis (see 100, 183, 213). If the malignant lesion is a carcinoma, it is usually a well differentiated thyroid carcinoma (39, 100, 228). Reticulum cell sarcoma and lymphosarcoma have also been reported (160, 164, 229).

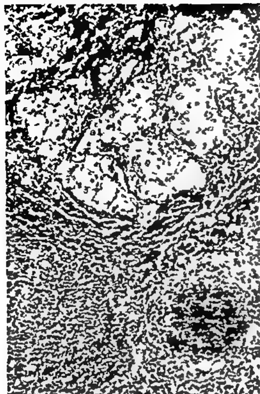
In this section the needle biopsy methods for cytological and histological examination and the cytological and histological findings in diffuse lymphoid thyroiditis will be compared. Furthermore, 2 patients with a coexisting malignant thyroid process and lymphoid thyroiditis will be described.

METHODS

Coarse needle biopsy of the thyroid for histological examination was performed by experienced surgeons with a Biegeleisen, Turner Warwick- or Silverman needle (Chapter II). Fine needle aspiration biopsy of the thyroid for cytological studies was done in the way described previously (page 9).

The histological diagnosis of diffuse lymphoid thyroiditis was based mainly on the recommendations of WOOLNER (226) and MASI et al. (132). According to Woolner, the fully developed or advanced stage of the disease is characterized by changes in follicular epithelium accompanied by a marked interfollicular infiltrate of plasma cells and lymphocytes. When these changes were apparently complete in all areas sampled the disease was referred to as diffuse thyroiditis (Examples Fig. 20, 21, 34, 35).

Histological examination of operative specimens from patients subjected to thyroid resection was originally included in the routine work of the department of pathology. Specimens were fixed in 10 % formalin. Two to seven sections were taken from each specimen and stained with haematoxylin - van Gieson and sometimes with haematoxylin-eosin. In the microscopical re-examination of



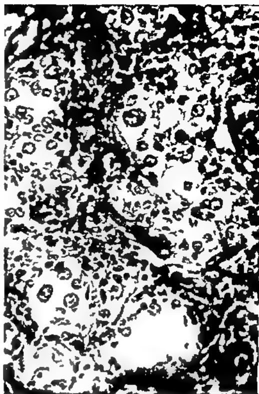


Fig 20—21 *Diffuse lymphoid thyroiditis (section File No 4438/63 HiG) Fig 20 (left) Photo micrograph showing lymphoid tissue fibrosis and fairly pronounced follicular epithelial polymorphism (× 105) Fig 21 (right) Detail of Fig 20 (× 262)*

the sections the lymphoid tissue, follicular epithelium + colloid and fibrosis were estimated from the areas they covered and at the same time the relative number of Askanazy cells was noted The following scale was used o = none + = scanty, + + = moderate, + + + = fairly abundant and + + + + = very abundant The degree of follicular epithelial polymorphism was graded in a similar way the corresponding symbols denoting none mild, moderate, fairly marked and very marked The estimation was based on an overall impression of the degree of polymorphism rather than on the most pronounced changes in a single cell

In the cytological evaluation of fine needle aspirates the same scale was used for estimation of the number of lympho reticular cells and follicular cells, and the amount of colloid and the degree of follicular epithelial polymorphism The number of Askanazy cells was judged as a percentage of 200 follicular epithelial cells in different counts of different parts of the smears Further details of the cytological examination as well as the

Table 12 *Histologically and cytologically evaluable material obtained by coarse needle or fine needle puncture of thyroid in patients with lymphoid or suspected lymphoid thyroiditis*

Biopsy method	Material obtained for evaluation			Total
	Abundant	Scanty	None or insufficient	
Coarse needle for histological examination	18	5	7	30
Fine needle for cytological examination	29	1	■	30

Difference between the two biopsy methods after pooling of groups scanty and none or insufficient $\chi^2 = 11.88$ $P < 0.001$

identification of Askanazy cells in MGG-stained smears are given in Chapter II

MATERIAL

The material consisted of 59 patients examined both histologically and cytologically and forming part of the series of 894 patients accounted for in Chapter III It embraced two groups, the first comprising 51 patients from whom needle biopsy specimens obtained both for histological and for cytological examination had shown a histological and/or a cytological picture consistent with the diagnosis of lymphoid thyroiditis In 29 patients of this group the thyroid was punctured first with the fine needle and later with the coarse needle for histological verification of the diagnosis of thyroiditis In 8 patients the order was the reverse and in 14 the thyroid was punctured with both the fine needle and the coarse needle at one and the same sitting To improve the comparability of the methods of biopsy the number of cases in which puncture was done on two occasions were made equal by randomly selecting 8 cases from the 29 in which the thyroid was first punctured with a fine needle This comparison was thus based on 30 cases

The other group consisted of 8 patients who had undergone partial or total thyroidectomy shortly—at most 4 months—after fine needle puncture and who had in the meantime not received cortisone thyroid preparation or thyrostatics

RESULTS

It is clear from Table 12 that in lymphoid thyroiditis fine needle biopsy produced evaluable material for cytological examination more often than biopsy with a coarse needle for histological examination and that the difference between the two biopsy methods ■ significant***

The histological diagnosis made from the samples obtained from 51 patients ■ given in Table 13 Diffuse lymphoid thyroiditis was diagnosed in 23 cases Scanty material with pictures histologically consistent with lymphoid thyroiditis was obtained in 11 cases In 2 of them

Table 13 *Histological diagnosis of material obtained by coarse needle biopsy from thyroid*

Diffuse lymphoid thyroiditis	23
Scanty material with appearances consistent with lymphoid thyroiditis	11
Follicular adenoma	1
No or insufficient thyroid tissue	16
Total	51

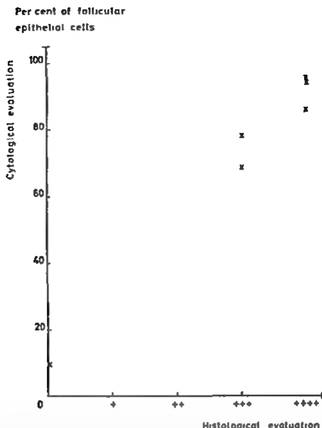


Fig 22 Comparison between the cytological and histological evaluation of number of Askanazy cells in 6 operated cases of diffuse lymphoid thyroiditis The number of Askanazy cells is given in percentage of 200 follicular epithelial cells counted in the cytological and in symbols (+) in the histological evaluation o = none + = scanty ++ = moderate +++ = fairly abundant ++++ = very abundant

as well as in the case with a follicular adenoma the picture was interpreted cytologically as focal lymphoid thyroiditis This patient was later operated upon with subtotal thyroidectomy Histological examination of the operative specimen showed a follicular adenoma and focal lymphoid thyroiditis in the adjacent thyroidal parenchyma

The results of the cytological examination of fine needle aspiration biopsy and the histological examination of surgical specimens from patients with diffuse lymphoid thyroiditis are compared in Fig 22—24 It is clear from Fig 22—23 that there was good agreement between the cytological and histological estimation of the number of Askanazy cells, lymphoreticular cells and follicular cells Agreement was also fairly good con-

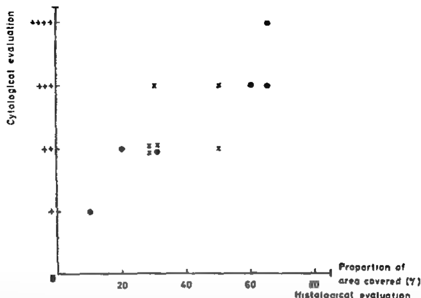


Fig 23 *Comparison between the cytological and histological evaluation of number of lymphoreticular cells (x) and follicular epithelial cells (●) in 6 operated cases of diffuse lymphoid thyroiditis The number of cells was estimated from the areas they covered in the histological, and in symbols (+) in the cytological evaluation The histological evaluation of the amount of epithelial cells included colloid For explanation of the symbols (+) see Fig 22*

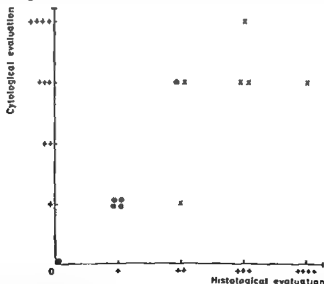
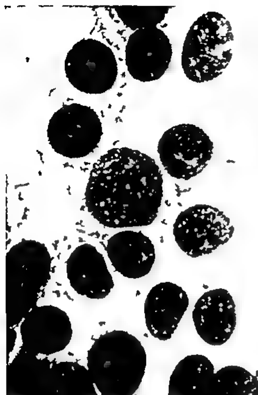


Fig 24 *Comparison between the cytological and histological evaluation of the amount of colloid (●) and degree of follicular epithelial polymorphism (x) in 6 operated cases of diffuse lymphoid thyroiditis o = none + = scanty or mild ++ = moderate +++ = fairly abundant or fairly marked ++++ = very abundant or very marked*



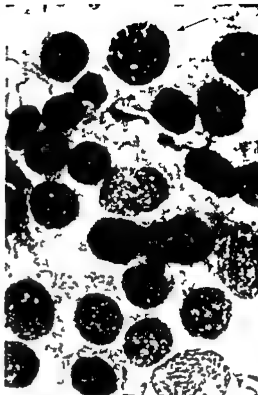


Fig 25—26 *Case 1 (A P File No 600/64 MGG) Cytolog al d a nosis Well d fferent ated thyroid carc noma F g 25 (left) Strongly prol ferat ng foll cular ep thel um w th n cle of var o s forms and s zes (× 1050) F g 26 (r ght) M tosis (arrow) of foll c lar ep thel m with prol ferat ve changes (× 1050)*

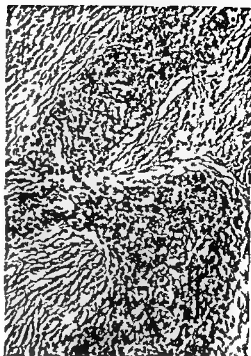
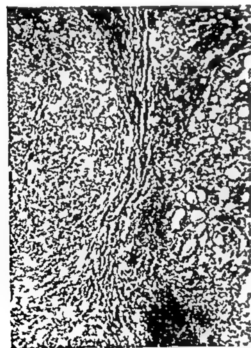
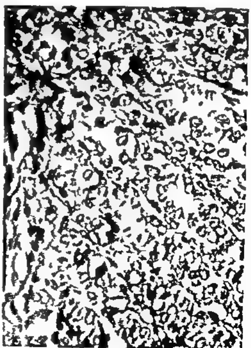
cerning the amount of colloid and the degree of follicular epithelial poly morphism (Fig 24)

Case reports Two cases of histologically or cytologically diagnosed lymphoid thyroiditis in association with a malig nant thyroid disease are reported below

Case 1 (A P) A previously healthy euthyro d woman, aged 55 had for 3 months not ced a hard egg sized lump at the s te of the left thyroid lobe [131]I test showed normal uptake and excret on A scint gram over the s te of the swelling showed a reduced and irregular uptake

Cytolog cal exam nat on (600/64 F g 25 26 60 61) Two asp rates were obtained from the swell ng but only one allowed evaluat on It conta ned abundant clusters of foll cular ep thelial cells with marked proliferative changes

Fig 27—30 *Case 1 (A P File No 9623/64) H stolog cal d agno s Strongly s spected well d fferent ated thyro d carc noma + focal lympho d thyro d t s F g 27 (top left) Area of f brosis w th ma nly sol d ep thel al format ons (HE × 26) F g 28 (top r ght) Deta l of adjacent area show ng f brosis and sol d ep thel al format ons (HE × 105) Fig 29 (bottom lef) Sol d and trabec lar ep thel al format ons adjacent normal thyro d t ss e and thyro d t ssue inf ltrated w h lymphoret cular cells (H G × 67) F g 30 (bot om r ght) Deta l of same spe m n show ng strongly prol ferat ng sol d ep thel al format ons (HE × 262)*

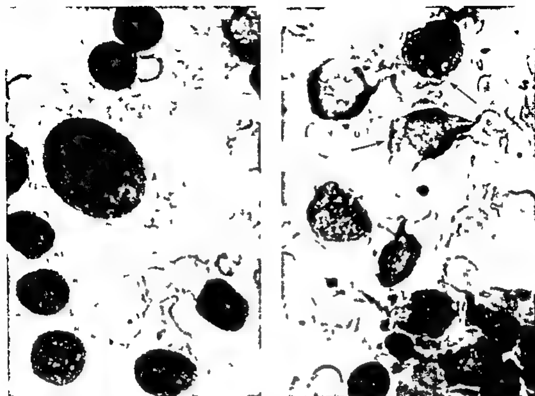


Fig 31—32 *Case 2 (E O File No 7_0/63 MGG) Cytological diagnosis Diffuse lymphoid thyroiditis with atypical lymphocytoid cells Fig 31 (left) Follicular cells of Askanazy cell appearance showing variation in size of nuclei abundant cytoplasm and faintly discernible cytoplasm granules (× 1050) Fig 32 (right) Atypical lymphocytoid cells with nuclei of different shapes and small round vacuoles (arrows) in cytoplasm (× 1050)*

and patches of considerable cellular atypia The nuclei were enlarged and of varying size shape and stainability (Fig 25 26 60 61) They had a loose chromatin network and 1—3 nucleoli sometimes large (Fig 61) A few mitotic figures were seen (Fig 26) The cytoplasm showed no paravacuolar granules No Askanazy cells and only scanty colloid were seen Scattered plasma cells but no signs of diffuse lymphoid thyroiditis

Cytological diagnosis Well differentiated thyroid carcinoma

At subsequent operation the thyroid lobes proved changed and the left lobe was the size of a hen s egg [illegible] thyroidectomy was done and several lymph nodes along the left recurrent nerve were removed

Histological examination (9623/64 Fig 27—30) The major part of the gland was finely nodular and in these areas there was a fair amount of colloid in others fairly abundant lymphoid tissue interstitially and some increase of the interlobular connective tissue The epithelium was mostly fairly low Only in very small areas were there any larger slightly picrinophilic granulated cells of Askanazy type In the left lobe adjacent a hazelnut sized necrotic region surrounded by granulation tissue undergoing fibrosis were markedly proliferating atypical epithelial cells which in usually solid or

trabecular formations seemed to pierce the connective tissue and grow into adjacent thyroid tissue. The picture strongly suggested well differentiated thyroid carcinoma. No ingrowth of vessels or lymph node metastases.

Histological diagnosis: Focal lymphoid thyroiditis + atypical follicular epithelial proliferations strongly suggesting well differentiated thyroid carcinoma.

Review in December 1967 revealed no signs of a local recurrence or metastasis.

Case 2 (E.O.). A woman aged 62 had for one year had mild neckpain and observed swelling of the neck. At examination she was euthyroid. The right lobe of the thyroid and the isthmus felt nodular and both were enlarged. ^{131}I test showed a 24 hour neck uptake of 13 % and a slightly irregular uptake without distinct cold nodulus in the scintigram.

Cytological examination (7_0/63. Fig. 31, 32, 62). A specimen from the right sided lump contained fairly abundant clusters of follicular epithelial cells. There was moderate cellular polymorphism and different sized, different shaped, large nuclei with abundant cytoplasm. All the follicular epithelial cells in segments with cellular polymorphism were Askanazy cells (Fig. 31). No paravacuolar granules and only scanty colloid were seen.

The lymphoreticular cells constituted most of the cells and were dominated by lymphocytoid cells. These showed cellular atypia with folded, different shaped nuclei (Fig. 3-) and sometimes nucleoli. Some of the cells had small round vacuoles in the cytoplasm (Fig. 32, 62). Stem cells of varying type, plasma cells and tissue bound, often phagocytising, reticulum cells completed the picture. The general cytological picture suggested diffuse lymphoid thyroiditis. Distinct atypia of the lymphocytoid cells was, however, a feature deviating from those of the conventional picture of thyroiditis and indicated further investigation for suspected malignant lymphoma.

Cytological diagnosis: Diffuse lymphoid thyroiditis with atypia of lymphocytoid cells.

The patient was subjected to surgical exploration and after exposure of the right thyroid lobe a small tumour like swelling was removed.

Histological examination (9252/63. Fig. 33–35). A hazelnut sized, firm and nodular, greyish tumour was found in one end of the biopsy specimen. Microscopical examination of this part showed an area rich in connective tissue and containing exuberant vegetations of a well differentiated papillary thyroid tumour (Fig. 33) infiltrating the neighbouring thyroid tissue. No ingrowth of vessels with certainty. But the epithelial atypia was so prominent and the mode of growth so proliferative and infiltrative that a diagnosis of papillary thyroid carcinoma was made. The rest of the thyroid parenchyma showed varying amounts of colloid and scattered small follicles poor in colloid, lined by high cubical epithelium with granular picrinophilic cytoplasm. Some nuclear polymorphism. Interstitially there was a varying degree of lymphoreticular infiltration, but without any appreciable atypia.

One week later total thyroidectomy was performed and the specimen was examined histologically (95-7/63). Despite previous partial thyroidectomy the specimen weighed as much as about 74 g. Macroscopically the cut surface was poor in colloid, partly fleshy and greywhite. Histologically this material contained abundant lymphoid infiltration and distinct Askanazy cells, i.e. a picture of diffuse lymphoid thyroiditis (Fig. 34). In several of the sections the reticular component was conspicuous and rich in mitotic figures. The reticulum cell nuclei showed prominent nucleoli but varied only slightly in size (Fig. 35). The histological sections did not allow any more detailed evaluation of the finer structure of the lymphoreticular cells. Malignant lymphoma was not seen in either specimen and thyroid carcinoma only in the first.

Histological diagnosis: Papillary thyroid carcinoma + diffuse lymphoid thyroiditis.

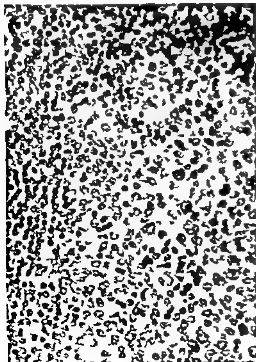
At review in April 1967 the patient showed no signs of a local recurrence or metastasis.

DISCUSSION

Both the histological and the cytological pictures reflect the morphology







of the organ But the two examination methods differ from one another in the information they can yield Cytological smears say nothing about the architecture of the structure which is one of the corner stones of the histological evaluation The cytological diagnosis is based on the identification of the various cells and the quantitative relationship between the types of cells The methods differ

Fig 33–35 Case 2 (E O File No 9252/63 95 7/63) Histological diagnosis Papillary thyroid carcinoma + diffuse lymphoid thyroiditis Fig 33 (top left) Part of papillary well differentiated thyroid carcinoma (HE 105) Fig 34 (top right) Another section showing pronounced lymphoid infiltration (HvG 26) Fig 35 (bottom) Detail of pre s ll stration showing tos s and mild nuclear atypia of reticulum cells Lymphocytoid cells apparently normal (HıG 262)

also in the procedure by which the material is obtained. A fine needle biopsy specimen contains an over-representation of loosely anchored cells and fluid material while connective tissue and vessels are very scanty or missing.

It is clear from Table 12 that in diffuse lymphoid thyroiditis fine needle puncture of the gland will more often yield acceptable material for cytological examination than will coarse needle biopsy for histological examination. This is because cytological examination needs less material than does histological examination and secondly because of technical advantages of the fine needle instruments, it being possible to puncture any part of the thyroid with the 0.6 mm thick needle without fear of complications.

In the histological sections of operated cases of diffuse lymphoid thyroiditis the amount of fibrosis, lymphoid tissue and follicles varied from one part of the specimen to another. Yet the agreement between the cytological and histological findings was good in all the 6 cases studied. In diffuse lymphoid thyroiditis the material obtained for cytological examination may thus be regarded as representative of the entire gland but as mentioned previously it says nothing about the amount of fibrosis if any.

In coexisting malignant thyroid process and lymphoid thyroiditis the histological (*e.g.* 30, 93, 95, 160, 229) as well as the cytological diagnosis (142, 198) is more difficult. This is illustrated in cases 1 (A.P.) and 2 (E.O.). In case 2 the 5×8 mm well differentiated papillary thyroid carcinoma had obviously not been punctured because the follicular epithelial cells in the smears were Askanazy cells and showed no features compatible with a diagnosis of papillary thyroid carcinoma. The increased number of immature and atypical lymphocytoid cells in the smears was a feature not occurring in the usual cytological picture of thyroiditis and suggested malignant lymphoma. In the histological sections there was a rich reticulum cell proliferation but no signs of malignant lymphoma. The histological routine sections did not allow any detailed evaluation of the finer structures of the cells, which are better seen in cytological smears (125). A strong lymphoreticular stem cell reaction sometimes seen in smears from lymph node metastases can mimic malignant lymphoma (198). The question as to relation between the immature and atypical lymphocytoid cells and the papillary thyroid carcinoma must be left open. But the cellular atypia of the lymphocytoid cell in the smears was such that surgical biopsy was decided upon. A specimen from the palpated lesion of the thyroid was removed and resulted in the unexpected discovery of a small papillary thyroid carcinoma.

As in case 2 a small thyroid cancer can be readily missed by puncture, and a well differentiated thyroid carcinoma *may sometimes be difficult to recognise* cytologically (28, 52, 198) and histologically (4). It should thus be stressed that a cytological finding of non-malignant cells in an aspirated biopsy specimen does not exclude the possibility of carcinoma.

In co-occurring thyroid carcinoma and lymphoid thyroiditis, the thyroiditis is often focal (121) As shown in Chapter III, such focal thyroiditis is detected in smears from fine needle aspirates in only about every fourth case of focal thyroiditis and then as a slight increase in the number of lymphoreticular cells The cytological diagnostic problems are thus confined mainly to recognisation of malignant thyroid cells

According to STEWART (193), in haematoxylin eosin stained smears and sections diffuse lymphoid thyroiditis is readily confused with small cell carcinoma of the thyroid In our experience, however, the cytological differentiation between these conditions usually causes no difficulties in MGG-stained smears studied under high magnification The cells of the small cell anaplastic thyroid carcinoma can owing to their polymorphism easily be distinguished from the mature lymphocytoid cells in lymphoid thyroiditis EINHORN & FRANZÉN (52), however, found the differentiation between this kind of carcinoma and malignant lymphoma impossible in MGG-stained smears

The pronounced follicular epithelial polymorphism, sometimes seen in smears from patients with lymphoid thyroiditis may lead the examiner's thoughts to malignant disease This happened in the preliminary examination of smears from 2 patients in the present thyroiditis series With increasing experience and on re examination of smears it was, however, realised that this cellular polymorphism was part of the formation of Askanazy cells

Hurthle cell carcinoma is sometimes seen in association with lymphoid thyroiditis (30) This combination did not occur in the present material but it would probably cause diagnostic difficulties in cytological examinations Mitoses of Askanazy cells (Hurthle cells) are seen in Hurthle cell carcinoma (126) but are extremely rare in lymphoid thyroiditis and in the present material it was seen in only 1 case The presence of mitoses of Askanazy cells may thus be a useful sign in the cytological diagnosis of coexisting Hurthle cell carcinoma and thyroiditis

Another diagnostic pitfall is a punctate from a lymph node metastasis of a well differentiated thyroid carcinoma, which may show a cytological picture liable to be mistaken for that of diffuse lymphoid thyroiditis But in such cases the Askanazy cells typical of thyroiditis are missing and if the person performing the biopsy gives a precise description of the site of the swelling it should be possible to avoid such errors

CHAPTER VI

INCIDENCE OF THYROID AUTOANTIBODIES AND ITS CORRELATION WITH CYTOLOGICAL FINDINGS IN LYMHOID THYROIDITIS IN ADULTS

By

P Sigvard Persson, Jonas Jonsson and Gunnel Biberfeld

Autoantibodies against four components of the human thyroid have been shown to occur particularly in thyroiditis Circulating antibodies against thyroglobulin have been demonstrated with the precipitation (168), latex aggregation and passive haemagglutination techniques (225) The two former reactions are less sensitive and demonstrate only high concentrations of anti thyroglobulin (0 1—0 01 μg of antibody nitrogen/ml) the latter reaction is very sensitive and demonstrates anti-thyroglobulin down to 0 001 μg of antibody nitrogen/ml (110 154, 192) Antibodies against a further component of the thyroid colloid the so-called second colloid antigen have been demonstrated with the immunofluorescence technique (8) Antibodies against thyroid cytoplasmic antigen have been demonstrated with the complement fixation test (166 207) and with the immunofluorescence technique (102) Antibodies to the cytoplasmic antigen seem to be identical with those demonstrable by the cytotoxic test (64 104 157) but apparently not with those reacting with organ specific antigen on the surface of thyroid cells in the mixed haemadsorption test (60, 108 109)

Thyroid autoantibodies in thyroiditis and other thyroid diseases have been the subject of several surveys (49, 72, 87 101 118, 167 170, 188)

The purpose of the study described in this chapter was to assess the incidence of thyroid antibodies in lymphoid thyroiditis diagnosed by fine needle biopsy of the thyroid The study was also extended to include an investigation of the effect of treatment with thyroid hormone on the incidence of antibodies and of the variation of the incidence of thyroid antibodies with the morphological picture of diffuse lymphoid thyroiditis

MATERIAL

Antigens

Thyroglobulin

Human thyroglobulin extracted with physiological saline from thyroid glands from cadavers and isolated by precipitation with ammonium sulphate (41) was used for coating tanned sheep erythrocytes employed in the passive haemagglutination test according to Borden (22)

Cytoplasmic thyroid antigen

Thyrotoxic thyroid glands obtained as soon as possible after thyroid resection were minced and homogenised together with 4 volumes of complement fixation buffer (see below) Coarse

tissue debris was removed from the homogenate by centrifugation for 10 minutes at 1500 g after which the homogenate was stored at −70°C and after standardisation used as antigen in the complement fixation test

Thyroid cultures

Human thyroid monolayer cultures were prepared from operative specimens The quality of the cultures was largely the same whether prepared from thyrotoxic or atoxic glands A suspension of dispersed thyroid epithelial cells was obtained by digestion of the tissue with 0.25 % trypsin A suspension of 4 million cells in 20 ml Hank s medium containing 0.5 % lactalbumin hydrolysate and 10 % normal calf serum was added to flasks with a flat bottom surface of 4.5 11 cm A continuous monolayer had usually grown out within 2–3 days incubation at +37°C Microscopical control after such incubation showed preponderantly epithelial like cells No cultures older than 5 days were used in the experiments reported below

Patients sera

The clinical material consisted of 87 patients with diffuse lymphoid thyroiditis 23 patients with focal or suspected diffuse lymphoid thyroiditis and a control series consisting of 43 patients with non toxic colloid goitre and 161 healthy persons of varying age without known thyroid disease

The group of diffuse lymphoid thyroiditis consisted of 87 of the 89 adults with cytologically verified diagnosis (Chapter III) The group examined included the sera from 56 patients treated with thyroid hormone usually l thyroxin 0.1–0.3 mg/day for 1 month to 5 years Of these patients 43 had been treated for more than 1 year Thirty-one patients had received no treatment at all Sera from the latter group was examined as a preliminary step in the diagnostic investigation and thus before the need for treatment had been considered The group was therefore not selected according to the severity of the condition and could thus be adequately compared with the treated group

Twelve patients with cytologically verified thyroiditis had clinical symptoms of hypo thyroidism a hard uneven goitre and abnormal serum protein findings (elevation of E S R hypergammaglobulinaemia increased alkaline phosphatase or positive thymol reaction) and were assigned to a group called clinical Hashimoto s disease

In the group with focal or suspected diffuse lymphoid thyroiditis the fine needle biopsy specimens from the thyroid showed the picture of focal lymphoid thyroiditis or consisted of scanty material the cytological picture of which was consistent with a diagnosis of diffuse lymphoid thyroiditis

The age and sex distribution within the group of patients with colloid goitre was the same as that of the group with diffuse lymphoid thyroiditis The diagnosis was based on the cytological picture of two needle biopsy specimens from different parts of the goitre The patients were euthyroid and had not been treated with thyroid hormone Neither did they have symptoms of rheumatoid arthritis diabetes mellitus pernicious anaemia or any other disease believed to be associated with diffuse lymphoid thyroiditis (see 14)

The control group of healthy persons consisted of 153 women of varying ages and 8 men The men and 87 of the healthy women were chosen so that they corresponded in age and sex to the patients with diffuse lymphoid thyroiditis and thus formed a separate group of matched controls

Serum for antibody examination by the immunocytological correlation studies was collected in association with the puncture of the thyroid The study of the plasma cells and thyroid antibodies for any intercorrelation was based only on those cases from which two thyroid punctates had been obtained

Reagent sera

Anti human globulin serum

Anti human globulin serum for the immuno fluorescence and mixed haemadsorption techniques was prepared by immunising sheep with electrophoretically purified human IgG The

serum obtained had a titre of 1:204 800 when tested against sheep erythrocytes sensitised with a subagglutinating dose of human anti-sheep erythrocyte serum.

When coating indicator cells for the mixed haemadsorption technique sheep erythrocytes sensitised with amboceptor were incubated at room temperature as a 1 % suspension with 1024 agglutinating units of the antiglobulin serum. For the immunofluorescence technique serum was conjugated with fluorescein isothiocyanate (163) and absorbed with calf and monkey liver powder. The serum then gave a titre of 1/25 600 when tested against sheep erythrocytes sensitised with a subagglutinating dose of human amboceptor. The serum was used diluted 1/10 in the immunofluorescence test.

Human amboceptor (human anti-sheep erythrocyte serum) for the inner layer of the indicator cells was kindly supplied by Dr K. Aho and Dr LEIKOLA, Helsingfors (2). When tested against an 0.2 % suspension of sheep erythrocytes and read from the sediment pattern in Widal tubes the serum had a titre of 1/1280. The inner layer of the indicator cells for the mixed haemadsorption technique was achieved by incubating sheep erythrocytes as a 1 % suspension with 6.4 agglutinating units of amboceptor.

METHODS

Passive heamagglutination test for demonstrating antibodies against thyroglobulin (TRC)

Sheep erythrocytes were treated with tannic acid and coated with thyroglobulin according to Boyden's technique (22). The concentration of the thyroglobulin used for coating was 100 µg/ml. The serum samples which were heated at +56 C for 30 minutes and absorbed with untreated sheep erythrocytes were diluted in twofold series in 0.5 ml volumes in Widal tubes and 0.05 ml of a 2 % suspension of thyroglobulin coated erythrocytes were added to each tube. The results were read from the sediment pattern of the erythrocytes after 18–24 hours at +20 C.

Complement fixation test for demonstrating antibodies against thyroid cytoplasmic antigen (CFT)

Antibodies against thyroid cytoplasmic antigen (207) were titrated with a microtechnique (180, 199). The serum samples were heated to +56 C for 30 minutes and diluted in twofold series in barbital buffer containing Ca and Mg ions. To 0.025 ml of serum dilution was added 0.025 ml of antigen solution (2 antigen units) and 0.025 ml of complement (fresh guinea pig serum, 2 complement units). After 1 hour at +37 C 0.05 ml of haemolytic system was added. After a further hour at 37 C the samples were placed in the cold at +4°C until non-lysed erythrocytes had sedimented. The last dilution which showed total absence of haemolysis was taken as the end point of the titration. The haemolytic system was prepared by mixing one volume of a 2 % suspension of sheep erythrocytes with one volume of a dilution of amboceptor containing four units per ml. This mixture was incubated for 1 hour at +37 C before use.

Immunofluorescence tests (IFL)

The indirect immunofluorescence technique (214) was used with frozen sections of thyrotoxic human thyroid glands. Antibodies against thyroid cytoplasm were demonstrated on acetone fixed sections and antibodies against the second colloid antigen on methanol fixed sections. The sections were incubated for 30 minutes at room temperature with a 5-fold dilution series of the serum sample after the latter had been heated at +56 C for 30 minutes. Antibodies thereby attached to structures in the sections were demonstrated by subsequent incubation for 30 minutes at room temperature with fluorescein labelled (163) anti-human globulin serum. The results were read in a Zeiss fluorescence microscope.

Mixed haemadsorption test for demonstrating antibodies against thyroid cell surface antigen (MH)

The application of this method (55, 59) to thyroid cultures was described earlier (108, 109).

tissue debris was removed from the homogenate by centrifugation for 10 minutes at 1500 g after which the homogenate was stored at −70 C and after standardisation used as antigen in the complement fixation test

Thyroid cultures

Human thyroid monolayer cultures were prepared from operative specimens The quality of the cultures was largely the same whether prepared from thyrotoxic or atoxic glands A suspension of dispersed thyroid epithelial cells was obtained by digestion of the tissue with 0 25 % trypsin A suspension of 4 million cells in 20 ml Hank s medium containing 5 % lactalbumin hydrolysate and 10 % normal calf serum was added to flasks with a flat bottom surface of 4 5 11 cm A continuous monolayer had usually grown out within 2−3 days incubation at +37°C Microscopical control after such incubation showed preponderantly epithelial like cells No cultures older than 5 days were used in the experiments reported below

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When coating indicator d
haemadsorption technique s
sensitised with ambocept at
room temperature as a h
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serum For the immunofl ase
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thiocyanate (163) and a and
monkey liver powder ve
a titre of 1/25 600 wh r eep
erythrocytes sensitised ting
dose of human amboc was
used diluted 1/10 in th ence
test

Human amboceptor (hum ythro-
cyte serum) for the inner icator
cells was kindly supp Dr K Airo
and Dr LEIKOLA H When
tested against an 0 2 sheep
erythrocytes and read ment
pattern in Widal tubes the serum titre
of 1/1280 The inner layer of the ind r cells
for the mixed haemad rption technique was
achieved by incubating the erythrocytes as
a 1 % suspension with 64 agglutinating units
of amboceptor

METHODS

Passive heamagglutination test for demonstrating antibodies against thyroglobulin (TRC)

Sheep erythrocytes were treated with tannic acid and coated with thyroglobulin according to Boyden s technique (22) The concentration of the thyroglobulin used for coating was 100 μg/ml The serum samples which were heated at +56 C for 30 minutes and absorbed with untreated sheep erythrocytes were diluted in twofold series in 0 5 ml volumes in Widal tubes and 0 05 ml of a 2 % suspension of thyroglobulin coated erythrocytes were added to each tube The results were read from the sediment pattern of the erythrocytes after 18 24 hours at +20 C

Complement fixation test for demonstrating antibodies against the cytoplasmic antigen (CFT)

Antibodies against thyroid gen (207) were titrated with a (180 199) The serum samples to +56 C for 30 minutes and series in barbital buffer cont Mg++ ions To 0 025 ml of added 0 025 ml of antigen units) and 0 025 ml of guinea pig serum 2 complem 1 hour at +37°C 0 05 ml of was added After a further samples were placed in the non lysed erythrocytes had last dilution which showed haemolysis was taken as the titration The haemolytic by mixing one volume of a 2 sheep erythrocytes with dilution of amboceptor per ml This mixture was at +37 C before use

Immunofluorescen

The indirect immu (214) was used w h toxic human thyroid thyroid cytoplasm acetone fixed section the second collo d sections The sectio minutes at room dilution series of the latter had been hea Antibodies there the sections wer s incubation for 3 d with fluoresc fre globulin serum zone a Zeiss fluorex t found

Mixed haem strating of thyroid x matched with colloid The ap thyroid without

Table 14 *Incidence of antibodies reacting against thyroid antigens in adult patients with lymphoid thyroiditis*

Diagnosis	No of patients tested	Incidence of: Thyroglobulin TRC ≧12.5	Thyroglobulin TRC ≧1600	Thyroid cell surface antigen MH ≧12.5 R	MH ≧12.5 F	MH ≧1600 R	MH ≧1600 F
Thyroiditis							
Diffuse lymphoid	87	53 (61)	25 (29)	52 (60)	33 (38)	44 (51)	9 (10)
Focal or suspected diffuse lymphoid	23	7 (30)	1 (4)	5 (22)	13 (57)	5 (22)	4 (17)
Controls (matched)							
Colloid goitre	43	8 (19)	1 (2)	*	17 (40)	0	1 (2)
Healthy persons	87	7 (8)	1 (1)	1 (1)	20 (23)	0	4 (5)

TRC = tanned red cell haemagglutination test
CFT = complement fixation test
MH = mixed haemadsorption test
IFL = immunofluorescence test
R = ring zone reaction
F = filled zone reaction
* = anticomplementary (±)

Monolayer cultures of thyroid epithelial cells were coated with a 4 mm thick layer of 0.75 % isotonic agar (Difco Bacto agar) to which 5 % of normal calf serum had been added Filter paper discs (Ford 80 pound filter paper) 5 mm in diameter soaked with serum dilution were placed on the agar layer and the flasks were allowed to stand with the agar layer uppermost for 48 hours at room temperature During this period the antibodies diffused from the filter paper discs through the agar layer and attached to the cultures where they formed circular zones The filter paper discs and the agar layer were afterwards removed and 13 ml of a 0.3 % suspension of indicator cells was added The indicator cells consisted in this case of sheep erythrocytes coated with an inner layer of human amboceptor and an outer layer of sheep anti human globulin The indicator cells were allowed to sediment on the cultures for 1–6 hours after which the supernatant was carefully decanted The indicator cells then remained adherent only in those areas where antibodies had been bound to the culture As shown by Espmark & Fagraeus (56) the correlation between the diameter of the haemadsorption zones and the logarithm of the antibody concentration in the test sera is linear The antibody content of the sera was therefore estimated from the size of the zones and expressed in the usual way as a titre In 63 titrations performed on different occasions with a standard serum the total variation of the titre was ± 1.5 twofold titre steps

Reactions in the serum dilution ≧ 1/12.5 in TRC and MH tests ≧ 1/10 in CFT and ≧ 1/5 in IFL test were regarded as positive

Cytological methods

The number of Askanazy cells and plasma cells was determined by differential count of 200 thyroid epithelial cells and 2000 lympho reticular cells respectively as described in Chapter II

Statistical methods

The statistical significance of the differences between the groups studied was calculated by the Student's t test and the χ^2 test In the χ^2 analysis Yates correction was used when the expected frequencies were below 1 (120) Other statistical operations used were analysis of variance linear regression analysis and the sign test and the Wilcoxon test of pair differences (190) In the regression analysis of the immunocytological correlation and in the calculation of the mean of antibody titres the titres were transformed to the exponent in the serum dilution series

and in controls Titre levels are given as reciprocals Figures within brackets represent incidence in per cent

reactions against						
Thyroid cytoplasmic antigen			Second colloid antigen		Positive by one or more tests	Positive in high titres by one or more tests
CFT	IFL		IFL			
≧10	≧5	≧25	≧5	≧25		
44 (51)	74 (85)	63 (7[illegible])	6[illegible] (71)	39 (45)	87 (100)	74 (85)
2 (9)	15 (65)	14 (61)	10 (43)	4 (17)	21 (91)	15 (65)
1 (2)[a]	26 (60)	1 ([illegible])	3 (7)	1 (2)	3[illegible] (74)	3 (7)
2 (2)	3 (3)	[illegible]	7 (8)	3 (3)	[illegible]8 (32)	6 (7)

High titres = TRC and MH ≧1600 CFT ≧10 IFL ≧25

RESULTS

Incidence of thyroid antibodies

The incidence of antibodies against various thyroid components in patients with diffuse lymphoid thyroiditis and focal or suspected diffuse lymphoid thyroiditis and in controls is given in Table 14 In the group with diffuse lymphoid thyroiditis antibodies against at least one antigen were found in all 87 cases Antibodies against thyroglobulin were demonstrated with the passive haemagglutination technique (TRC) in 53 cases (61 %) and 25 (29 %) had an anti thyroglobulin titre of 1/1600 or more Antibodies to cell surface antigen were demonstrated with the mixed haemadsorption technique (MH) in 85 cases (98 %) and in 53 (61 %) the titre was 1/1600 or more Two different types of reaction so-called ring and filled zone reactions (109) were obtained in this test The ring zone reaction was the predominant reaction in diffuse lymphoid thyroiditis particularly among the cases with high titres Antibodies against cytoplasmic antigen were demonstrated in 74 cases (85 %) with the immunofluorescence technique (IFL) and in 44 cases (51 %) also with the complement fixation technique (CFT) Antibodies against the second colloid antigen were demonstrated with the immunofluorescence technique in 62 cases (71 %) Antibodies in high titres (*viz* TRC and MH ≧ 1/1600 CFT ≧ 1/10 IFL ≧ 1/25) were found by one or more tests in 74 cases (85 %)

The incidence of thyroid antibodies was lower in the group with focal or suspected diffuse lymphoid thyroiditis than in the group with diffuse lymphoid thyroiditis The ratio between the frequencies of ring respectively filled zone reactions was the inverse of that found in diffuse lymphoid thyroiditis

The incidence and titres of thyroid antibodies in the age and sex matched control groups of patients with colloid goitre and of healthy persons without

Table 15 *Incidence (%) of antibodies reacting against thyroid antigens in female patients with diffuse lymphoid thyroiditis and apparently healthy females of different age groups Titre levels are given as reciprocals The same abbreviations as in Table 14*

Diagnosis	Age group	No of patients tested	Incidence (%) of reactions against: Thyroglobulin TRC ≥12 5	TRC ≥1600	Thyroid cell surface antigen MH ≥12 5	MH ≥1600	Thyroid cytoplasmic antigen CFT ≥10	IFI ≥5	IFI ≥25	Second colloid antigen IFL ≥5	IFL ≥25
Diffuse lymphoid thyroiditis	20—39	18	66 6	11 1	100	38 9	27 8	77 8	55 6	61 1	27 8
	40—59	46	54 3	26 1	97 8	60 9	52 2	84 8	76 1	76 1	47 8
	60—79	15	60 0	33 3	93 3	66 7	73 3	86 7	66 7	80 0	60 0
Healthy females	20—39	39	7 7	0	5 1	0	0	0	[illegible]	10 3	2 6
	40—59	36	8 3	2 8	2. 2	5 6	2 8	11 1	0	11 1	2 8
	60—79	77	14 3	1 3	42 9	13 0	2 6	11 7	1 3	14 3	[illegible]

Table 16 *Incidence of antibodies reacting against thyroid antigens in adult patients with clinical Hashimoto s within brackets represent incidence in per cent The same abbreviations as in Table 14*

Diffuse lymphoid thyroiditis	No of patients tested	Incidence of: Thyroglobulin TRC ≥12 5	TRC ≥1600	Thyroid cell surface antigen MH ≥12 5	MH ≥1600
Clinical Hashimoto s disease	1.	9 (75)	5 (42)	12 (100)	9 (75)
Cytologically diagnosed (= total thyroiditis)	87	53 (61)	25 (29)	85 (98)	53 (61)

Difference between incidence of antibodies in clinical Hashimoto s disease and cytologically

Table 17 *Incid nce of thyroid antibodies in patients with diffuse lymphoid thyroiditis treated with thyroid lymphoid thyroiditis examined before and after 4—18 months treatment with thyroid hormone Titre levels are Table 14*

Groups	No of patients tested	Incidence of: Thyroglobulin TRC ≥12 5	TRC ≥1600		Thyroid cell surface antigen MH ≥12 5	MH ≥1600
Not treated	31	16 (52)	9 (29)	5 (16)	30 (97)	16 (52)
Treated	56	37 (66)	27 (48)	20 (36)	55 (98)	37 (66)
Before treatment	14	5	3	2	14	7
After treatment	14	7	4	1	14	9

Difference in frequency of negative and positive reactions in the various tests between the groups
Difference in the mean of the positive titres in TRC MH and CFT and in the frequency of positive

known thyroid disease were much lower than in the two previously mentioned groups (Table 14) With the exception of the filled zone reaction in the MH test the difference between the incidences of the various thyroid anti bodies in patients with diffuse lymphoid thyroiditis on the one hand and in patients with colloid goitre and in healthy persons on the other was significant*** at both the titre levels examined The reactions with thyroglobulin in the TRC test with cyto plasmic [illegible] in the IFL test and the filled zone r[illegible] in the MH test were, [illegible]ever ob[illegible]ed so often in the controls [illegible] diagnostically relevant differences against the thyroiditis group were obtained only at the titre level ≧ 1/1600 for TRC test MH test and ≧ 1/25 for IFL

The ring zone reaction in MH test occurred in only one case in the control groups

Table 15 shows the incidence of thyroid antibodies in women of different age groups with diffuse lymphoid thy-

disease and cytologically diagnosed diffuse lymphoid thyroiditis Titre levels are given as reciprocals Figures

reactions against					Positive in high titres by one or more tests
Thyroid cytoplasmic antigen			Second colloid antigen		
CFT	IFL		IFL		
≧10	≧5	≧25	≧5	≧25	
8 (67)	12 (100)	10 (83)	9 (75)	5 (42)	12 (100)
44 (51)	74 (85)	63 (7-)	62 (71)	39 (45)	74 (85)

diagnosed thyroiditis (χ^2 analysis) N S D

hormone (l thyroxin 0 1 – 0 3 mg/day) and not treated with the hormone and in 14 patients with diffuse given as reciprocals Figures within brackets represent incidence in per cent The same abbreviations as in

reactions against					
Thyroid cytoplasmic antigen				Second colloid antigen	
CFT		IFL		IFL	
≧10	≧80	5	≧25	≧5	≧25
15 (48)	6 (19)	25 (81)	21 (68)	23 (74)	15 (48)
29 (52)	9 (16)	49 (87)	42 (75)	39 (70)	24 (43)
7	3	12	9	11	7
8	0	9	8	12	7

not treated and treated and between the groups before and after treatment (χ^2 analysis) N S D
IFL titres between the groups not treated and treated (t test respectively χ^2 analysis) N S D

Table 19 *Incidence of antibodies against thyroglobulin and cytoplasmic antigen in patients with diffuse lymphoid thyroiditis reported by various authors*

Author	Total No of patients tested	No of females tested	Incidence of reactions against					
			Thyroglobulin (TRC)			Cytoplasmic antigen (CFT)		
			Titre ≥	No of positive	Per cent positive	Titre ≥	No of positive	Per cent positive
Paine et al (1957)	32	?	1/9	14	44			
Roitt & Doniach (1958)	106	?	1/5	96	91	1/5	96	91
Owen & Smart (1958)	78	?	1/10	63	81			
Cline et al (1959)	16	15	1/10	14	[illegible]			
Belyavin & Trotter (1959)	64	?				1/5	51	80
Fahey & Goodman (1960)	11	?	1/10	10	91			
Mackey & Perry (1960)	11	?	1/20	10	91			
Hackett et al (1960)	8	?	1/10	6	75			
Fulthorpe et al (1961)	303	279	1/5	251	83	1/4	260	86
Porter & Fennell (1961)	13	13	?	7	54	?	9	69
Leboeuf et al (1961)	31[a]	27	1/5	22	71	1/4	14[b]	52
Skanse & Nilsson (1961)	15	?	1/5	11	73	1/5	13	87
Rawstron & Farthing (1962)	30	?	1/2500	25	83			
Saxena & Crawford (1962)	17[a]	15	1/10	16	94			
Senhauser et al (1962)	66	?	?	55	83			
Jaffiol et al (1964)	10	?	1/25	6	60			
Decourt et al (1964)	29	28	1/20	27	93			
Witebsky & Rose (1964)	127	?	1/1	82	65			
Beha et al (1965)	11	?	?	10	91	?	8	73
Thomas et al (1965)	13	?	1/10	6	46	1/2	4	31
Doniach et al (1965)	38[a]	31	1/5	18	47	1/4	24	63
Buchanon & Harden (1965)	63	58				?	58	92
Halberg (1965)	37	?	?	34	92	?	31	84
Anderson et al (1967)	107	?	1/5	88	82	1/5	31[c]	62
Persson et al (1967)	87	79	1/12.5	53	61	1/10	44	51

[a] = juvenile thyroiditis [b] = 27 patients examined [c] = 50 patients examined

(IFL 85 % CFT 51 %) found in the material reported here was probably due to the diagnosis of thyroiditis being based mainly on the findings made at cytological examination. Fine needle biopsy is a very small operation and could therefore be used liberally in the investigation of goitre and/or thyroid functional disorders whether thyroiditis was suspected or not. The material used for the present study was therefore not so restricted selected as that in most other series where the diagnosis of thyroiditis was based on clinical symptoms or on histological examination of material removed at strumectomy or at biopsy. A similar difference has also been observed previously in children and adolescents. Leboeuf & Ducharme (115) in a survey of juvenile thyroiditis commented on the lower frequency of antibodies against thyroglobulin (TRC) and thyroid cytoplasmic antigen (CFT) in a Swedish material (46) of cases diagnosed mainly by fine needle biopsy (144) as compared with two American

series (113 173) and felt that this might be due to constitutional differences between the Swedish and the American populations It is, however, probable that DONIACH NILSSON & ROITT's (46) Swedish material of children, like that in the present investigation of adults was less rigidly selected for reasons mentioned above The higher incidence and titre of antibodies in the group of 'clinical Hashimoto s disease support this assumption

A general evaluation of the mixed haemadsorption reaction in the diagnosis of thyroid disease will be published elsewhere (108) With this technique two types of reaction are demonstrated (109) one of which, the ring zone reaction was much more common in diffuse *lymphoid thyroiditis than the other type* the filled zone reaction (Table 14) The ring zone reaction appears to provide a possibility of distinguishing lymphoid thyroiditis from colloid goitre also at a low titre level (Table 14)

Table 15 shows an increase in the incidence and titre of thyroid antibodies with increasing age both in patients and controls This is in agreement with the results of previous studies (46) of thyroid antibodies in Swedish children and adolescents with mainly cytologically diagnosed diffuse lymphoid thyroiditis (144) Antibodies against one or more of the thyroid antigens studied (thyroglobulin, cytoplasmic antigen second colloid antigen) were demonstrated in all these patients Antibodies against thyroglobulin and thyroid cytoplasmic antigen were however demonstrated less often and in lower titres than reported for adults with thyroiditis The incidence of thyroid antibodies in corresponding controls consisting of healthy children and children with colloid goitre was nil and 8 % respectively so that at this age the occurrence of thyroid antibodies, even in low titre, was diagnostically highly significant In higher age groups however thyroid antibodies in low titre appeared with such frequency in healthy women that only high antibody titres can support the diagnosis of lymphoid thyroiditis (Table 15) This is in accord with previous studies (*e g* 49) In the present material titre levels of diagnostic value may be set at 1/1600 for the TRC and MH reactions, at 1/25 for IFL reactions and at 1/10 for the CFT At these levels *thyroid antibodies were demonstrated* in 7 % or less of the controls consisting of healthy women and patients with colloid goitre (Tables 14 and 15) It should however be stressed that with the possible exception of the ring zone reaction in the MH test the difference between the frequency of antibodies in thyrotoxicosis and diffuse lymphoid thyroiditis is even at these titre levels hardly large enough to be of value in the differential diagnosis between the two conditions (108)

OWEN & SMART (147) found a lower frequency and titre of antibodies in the TRC test in a group of patients treated with thyroid hormone more than one year than in those untreated or treated for less than one year A decrease of the titre in the TRC test and the CFT during thyroid hormone treatment has been reported (115) SKANSE & NILSSON

(182) could not verify OWEN & SMART's results and felt that the decrease of TRC titre found by the latter authors was probably due to a cessation of the autoimmune process rather than to any effect of therapy. The results of OWEN & SMART may also have been affected by the fact that almost half of their patients had been operated upon with partial thyroidectomy, which can reduce the incidence of antibodies (47). In the material studied in this report the antibody titre against thyroid cytoplasmic antigen in the IFL test was reduced significantly** during treatment with thyroid hormone. If the observed change was caused by the treatment, a corresponding effect should be expected to appear in the comparison between the treated and untreated groups. In these groups, however, a slight, not significant *increase of the incidence and titres of* antibodies against cytoplasmic antigen was noted among the treated patients. The differences in the incidence and titres of all other thyroid antibodies were not significant. These findings thus do not support the assumption that treatment with thyroid hormone will affect the occurrence and titre of thyroid antibodies in patients with diffuse lymphoid thyroiditis, but show clearly that the lower frequency of thyroid antibodies in our series compared with series reported by other authors (Table 19) cannot be explained by the fact that most of the patients in our material were receiving thyroid hormone at the time of determination of the antibody titre.

The correlation between the occurrence of thyroid antibodies and thyroid histology has previously been the subject of a number of investigations. A positive correlation has been demonstrated between the incidence of antibodies against thyroglobulin and thyroid cytoplasmic antigen and focal lymphoid hyperplasia (11, 26, 43, 76, 106, 174, 178). The incidence of thyroid antibodies increases with the extent of the focal lymphoid thyroiditis (11, 26, 43, 178) and antibodies against one or more thyroid components can, as in our material, be demonstrated in all cases of diffuse lymphoid thyroiditis (*e.g.* 49). Attempts were made to correlate the incidence of thyroid antibodies with morphological details in the microscopical picture of the thyroid. A significantly* higher mean relative number of Askanazy cells was found in the group with demonstrable antibodies against the second colloid *antigen and against cytoplasmic antigen* in the CFT and IFL test than in the group without antibodies against these antigens. However, a formal correlation between the relative number of Askanazy cells and antibody titres could not be demonstrated. The association found is compatible with DONIACH's (43) assumption that the hypercellular variant of thyroiditis with epithelium of Askanazy cell appearance (178) is associated with high titre in the CFT. Whether the association between the incidence of antibodies against thyroid cytoplasmic antigen and the number of Askanazy cells implies an effect of the antibodies on the Askanazy cells or the antibodies may develop because of a particularly copious leakage of antigens from these cells which are characterised by an abundant

cytoplasm with unusual staining properties and a high content of mitochondria (e g 86 97, 171) could not be decided from the evidence available

SENHAUSER (178) reported a high titre of antibodies against thyroglobulin (TRC) in the fibrous variant of Hashimoto s struma (93) which besides marked fibrosis often has a large number of plasma cells (178) No correlation was found between the incidence of thyroid antibodies and the relative number of plasma cells in the thyroid in the present study This can be explained by the fact that also lymphocytoid cells and extrathyroid plasma cells take part in the formation of antibodies The correlation between the extent of lymphoreticular infiltrates in the thyroid and the occurrence of thyroid antibodies suggests that these cells may take part in the antibody formation More probably, however, they mainly reflect the degree of cellular immunity

CHAPTER VII

CLINICAL OBSERVATIONS IN PATIENTS WITH DIFFUSE LYMPHOID THYROIDITIS WITH SPECIAL REFERENCE TO THE EFFECT OF TREATMENT WITH THYROID HORMONE

By

Peter Heimann, P Sigvard Persson and Lars Risholm

The clinical picture and laboratory findings in diffuse lymphoid thyroiditis are well known and have been described in fair detail by HAZARD (93) and SKILLERN (183), for example This section on diffuse lymphoid thyroiditis diagnosed by cytological examination of fine needle biopsy specimens will therefore deal only with some symptoms and signs of the disease and with the effect of treatment with thyroid hormone on size of goitre

MATERIAL AND METHODS

The series consisted of 125 cases of diffuse lymphoid thyroiditis diagnosed by cytological examination of fine needle biopsy specimens from the total series of 894 patients subjected to puncture biopsy of the thyroid (Chapter III, p 18) A clinical investigation of most of the 36 patients with juvenile thyroiditis has been described previously by NILSSON & DONIACH (143) Therefore, apart from the age and sex distribution the analysis was limited to observations made among the 89 patients who were above 19 years of age As the patients were examined clinically by different physicians the laboratory tests used were not the same in all cases

Examination with radioiodine was performed at the same time as the thyroid puncture in 53 cases The patients were given 20 μC ^{131}I and 7, 24 and 48 hours later the uptake by the thyroid gland was measured in the conventional way with a scintillation counter

Changes in the size of goitre were noted in 62 adults with diffuse lymphoid thyroiditis treated with thyroid hormone, in 15 adults with untreated diffuse lymphoid thyroiditis, and in 62 controls with non toxic colloid goitre treated with thyroid hormone Twelve adult patients with diffuse lymphoid thyroiditis were excluded from this part of the investigation, because 3 had been operated upon without previous treatment, 3 had been treated with combined thyroid hormone and thiamazole or corticosteroids 1 could not be traced for the follow up and 5 had no goitre, or the size of the goitre at the beginning of treatment had not been recorded The controls were sex and age matched with the patients with treated diffuse lymphoid thyroiditis and randomly selected from

a material of colloid goitre diagnosed by cytological or histological examination of needle biopsy specimens

As a rule patients with diffuse lymphoid thyroiditis and the controls with colloid goitre were treated with l thyroxin in a dose of 0.05 to 0.30 mg/day In both groups the commonest dose used was 0.15—0.20 mg/day A few patients were given desiccated thyroid instead of l thyroxin Thyroid hormone was given because of hypothyroidism moderate or large goitre or local symptoms of the goitre

At frequent follow up the goitre was palpated and the circumference of the neck measured for any change in the size of the thyroid The size of the goitre in patients with thyroiditis was usually judged independently by two examiners in patients with colloid goitre by one A change in the size of the goitre was accepted as such only when the increase or decrease was unequivocal and in the patients examined by two of the authors only when there was an agreement of the results The patients were followed up for 10 to 48 months

RESULTS

Cytological examination of fine needle biopsy specimens of the thyroid from 894 patients revealed diffuse lymphoid thyroiditis in 125 (14 %)

The age and sex distribution of the 125 patients with diffuse lymphoid thyroiditis is given in Table 20 Of the 125 patients 114 (91.2 %) were females Fig 36 compares the age distribution of the thyroiditis series with that of the entire series (894 patients) in which puncture had been performed The age distribution was largely the same in both series with the exception of the 10—19 year class where diffuse lymphoid thyroiditis was more common

The erythrocyte sedimentation rate (E.S.R.) was measured in 63 patients at the time of the puncture of the thyroid and was found to be less than 20 mm/1 hour in one third of the cases and between 20 and 50 mm in 32 (50 %) In 10 it exceeded 50 mm/1 hour

The ratio between the number of Askanazy cells in per cent of 200 follicular epithelial cells counted and the maximum ^{131}I uptake by the thyroid within 7, 24 and 48 hours in 53 patients with diffuse lymphoid thyroiditis are given in Fig 37 The largest uptake was usually found after 24 hours There was a significant** negative correlation between the relative number of Askanazy cells and the largest ^{131}I uptake

During treatment with thyroid hormone the goitre decreased in 53 (85 %) of 6 patients with diffuse lymphoid thyroiditis (Table 21) The decrease in the size of the goitre was noted within the first 2—3 months In the control

Table 20 *Age and sex distribution of patients with diffuse lymphoid thyroiditis diagnosed cytologically*

Age	Females	Males	Total
10—19	33	3	36
20—29	9	0	9
30—39	10	[illegible]	10
40—49	27	3	30
50—59	19		1
60—69	13	1	14
70—79	3		3
Total	114	11	125

and/or thyroid functional disorders The patients were living in a costal district, where nothing suggests dietary iodine deficiency and endemic goitre This may explain the rather high frequency of diffuse lymphoid thyroiditis in the patients studied

The high frequency of diffuse lymphoid thyroiditis in children and adolescents has been pointed out previously by SAXENA & CRAWFORD (173), LEBOEUF & BONGIOVANNI (113) and NILSSON & PERSSON (144) As is clear from Fig 36, diffuse lymphoid thyroiditis was most common in the 10—19 year age group No explanation can be offered for the higher frequency of diffuse lymphoid thyroiditis in this age group It may, perhaps, be due to differences between the selection of children and adolescents and that of adults In the present material, as in other thyroiditis series (see 93, 183) the disease is much more common in females than in males

Elevation of the E S R is common in diffuse lymphoid thyroiditis (24, 44, 71, 85) This was also confirmed in our material It might be mentioned that in 2 patients thyroiditis was discovered in association with an investigation of the cause of elevated E S R In both patients thyroiditis proved to be the cause of the elevation

A firm goitre with an irregular surface was one of the most important palpatory findings The more that our interest was focused on thyroiditis, the better our possibilities of predicting or suspecting diffuse lymphoid thyroiditis on the basis of palpatory findings But even then the cytological diagnosis of thyroiditis was unexpected in 1/3 to 1/2 of the cases

In patients with a reduced uptake of ^{131}I the percentage of Askanazy cells was significantly** higher than in patients with normal or increased uptake (see Fig 37) Patients with reduced ^{131}I uptake in our material had clinical symptoms or laboratory findings suggesting hypothyroidism and an impalpable or only moderately enlarged thyroid There is thus reason to suspect the occurrence of hypothyroidism if the follicular epithelial cells are Askanazy cells and the needle biopsy specimen is taken from an impalpable or only moderately enlarged thyroid

Treatment with thyroid hormone results in a regression of the goitre in patients with diffuse lymphoid thyroiditis (see 7) Thus, in 134 (85 %) of 158 cases of diffuse lymphoid thyroiditis collected from the literature by ASTWOOD et al (7) in 1960, regression of the goitre was noted during treatment with thyroid hormone Such regression was also observed in our material Table 22 shows that adequate doses of thyroid hormone, *i e*, l thyroxin in a daily dose exceeding 0 1 mg is necessary to produce a definite regression of the goitre

GREER & ASTWOOD (78), LAMBERG et al (112) and ASTWOOD et al (7) found treatment of diffuse and nodular atoxic goitre with thyroid hormone to produce regression of the goitre in more than half of the cases In their series, however, goitre was not examined morphologically so that some of the patients might have had lymphoid thyroiditis In our control series of cytologically or histologically diagnosed colloid goitre, the goitre

regressed in less than half of the patients and less often than in the thyroiditis series (Table 21)

Thyroid carcinoma has been reported with a frequency of 1—12 % in lymphoid thyroiditis series (see 100 183 213) The cause of the divergency of the results has been discussed by CRILE JR & HAZARD (38) for example, who claimed that the frequency of carcinoma is higher in series including focal lymphoid thyroiditis If the case with thyroid carcinoma and focal thyroiditis (case No 1, page 50) is included it would mean that there were 2 cases (1 6 %) of malignant thyroid process in our thyroiditis series Clinical and cytological follow up in the other cases revealed no further case of malignant thyroid disease Neither did CRILE JR & HAZARD (38) find any case of thyroid carcinoma at follow-up of their series of diffuse lymphoid thyroiditis

CHAPTER VIII

GENERAL DISCUSSION AND CONCLUSIONS

As pointed out in Chapter I, it now appears to be generally accepted that acute suppurative, subacute, Riedel's and lymphoid thyroiditis are four different entities and that the first three of them are clinicopathologically well defined diseases. Nevertheless, in certain phases these diseases are sometimes difficult to distinguish from one another on the basis of clinical and laboratory findings only. Furthermore other diseases of the thyroid can simulate thyroiditis. It is therefore obvious that a simple, quick and reliable method for establishing a differential diagnosis of these diseases would be welcome.

Fine needle aspiration biopsy specimens of the thyroid of patients with acute suppurative, subacute or diffuse lymphoid thyroiditis show characteristic cytological features readily distinguished from one another and from those seen in the single case of Riedel's thyroiditis studied (Chapter III). This means that provided the material aspirated is sufficient for cytological evaluation the first three forms of thyroiditis can be readily diagnosed by microscopical examination of smears. A positive diagnosis of focal lymphoid thyroiditis on the other hand was possible in only one fourth of the cases in which fine needle biopsy specimens had been obtained.

Diffuse lymphoid thyroiditis has formerly been regarded as a rare disease and has been diagnosed only in patients with the classical clinical picture of Hashimoto's disease, *i.e.*, a middle aged woman with a firm goitre, myxoedema and abnormal serum protein findings or by histological examination of tissue obtained at strumectomy. The introduction of histological examination of puncture biopsy specimens of the thyroid marked a praiseworthy advance in the diagnosis of thyroiditis (12, 57, 89, 95). But the material obtained by such a procedure is often not sufficient to allow a firm histological diagnosis (89, 95, 98, 172, 215), as was apparent also from the present investigation. It was thus found (Chapter V) that in lymphoid thyroiditis it was more often possible to obtain evaluable material for diagnosis by fine needle puncture for cytological examination than by coarse needle puncture for histological examination. The difference between the methods was significant***.

The discovery, by ROITT et al. (168) of precipitins "against" thyroglobulin in

serum of patients with diffuse lymphoid thyroiditis, and the induction, by WITEBSKY et al (169, 223), of thyroiditis in rabbits prompted numerous investigations by experimental immunologists and clinicians It was hoped that the determination of circulating antibodies could be exploited to facilitate the diagnosis and the understanding of the pathogenesis of the disease These hopes have, however so far proved unfounded It is still not known whether the thyroid antibodies are directly correlated with the cause of thyroiditis or are only a secondary phenomenon Only when present in high concentration most of the thyroid antibodies are evidently of any substantial value in the diagnosis of diffuse lymphoid thyroiditis In the evaluation of the results of the determination of thyroid antibodies consideration must be given to the type of antibody, the sensitivity of the reactions used and the age and sex of the patient In a slightly selected series of diffuse lymphoid thyroiditis like that in the present investigation the number of cases with low titres of thyroid antibodies is increased in comparison with the results of previous authors (for references see Chapter VI, p 66) As shown by DONIACH et al (46) and also found in the present investigation (Table 15 Chapter VI), in the low age classes even low titres are of diagnostic value As a screening method to discover diffuse lymphoid thyroiditis in adults however determination of thyroid antibodies is insufficient

Cytological examination *i e* fine needle puncture of the thyroid and light microscopical examination of the aspirated material also means a further advance in the diagnosis of diffuse lymphoid thyroiditis Puncture is only a minor procedure and is safe, it can be used liberally Though only little material therefore is necessary for cytological examination and though the histological picture of diffuse lymphoid thyroiditis can vary from one part of the gland to another comparative investigations (Chapters II and V) showed that the aspirated material may be regarded as representative of the entire gland regarding the amount of lymphoreticular cells follicular epithelial cells and colloid and degree of epithelial polymorphism The amount of connective tissue, on the other hand cannot be judged in cytological specimens With the above mentioned method diffuse lymphoid thyroiditis was diagnosed in 14 % of all patients from whom biopsy specimens had been aspirated The results presented suggest that fine needle biopsy of the thyroid is at present the best screening method for diagnosing diffuse lymphoid thyroiditis in patients with thyroid diseases

The hypothesis of the biological gradient of diffuse lymphoid thyroiditis and the way in which it can be diagnosed are best illustrated by the work of MASI et al (Fig 38) (131) In the original figure is inserted the cytological method which embraces not only the medically or surgically detected cases but also some of those cases not diagnosed before autopsy It is also clear from the figure that the selection of cases of diffuse lymphoid thyroiditis varies with the

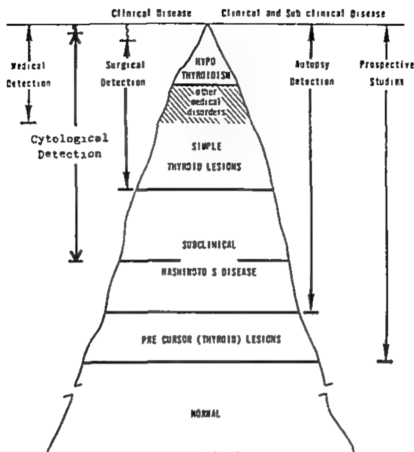


Fig 38 *Diffuse lymphoid thyroiditis (Hashimoto s disease)—its biological gradient and detection The cytological detection is inserted in the original figure which is reproduced from* MASI et al (131) *by courtesy of the Publishers of J Chronic Diseases*

diagnostic method used Published series of thyroiditis therefore vary in their composition which may explain the differences in antibody findings results of thyroid function tests and clinical features, reported by various authors

Histological examination of the thyroid in autopsy series has shown that the incidence of diffuse lymphoid thyroiditis increases with age (130) Our results, like those of others (113, 143, 173) show that diffuse lymphoid thyroiditis is common also among young persons with goitre With the duration of thyroiditis or with the patient s age the morphological picture changes The relative number of Askanazy cells and plasma cells increases and the relative number of stem cells decreases (Fig 9 16 19 and Table 8 Chapters III and IV) In the

lower age classes Askanazy cells are missing or very sparse they increase with age and after the age of 40 they usually dominate the picture of the follicular epithelial cells (Chapter III) Since the occurrence of Askanazy cells was an obligatory condition for the "classical" histological diagnosis Hashimoto's struma (91, 93), this diagnosis was automatically reserved for the middle and higher age classes The new pathoanatomical term diffuse lymphoid thyroiditis, which covers both oxyphilic and non oxyphilic epithelium (49, 178, 226, 228) appears to be a more suitable term

The Askanazy cells are follicular epithelial cells with characteristic morphology Their appearance in cytological and histological specimens and their synonyms (Hurthle cells oncocytes oxyphilic epithelium) have been discussed in Chapter II They have been subjected to many studies but neither their function nor the exact mode of their development are known They have been described as follicular epithelial cells that have undergone regression (117), are involuted or 'burned out' (69) developed by the effect of thyroid antibodies with cytotoxic effect (*e g* 48) misdirected in their thyroglobulin production (1-2) or involved in calcitonin production (65)

Histochemical studies have shown that the Askanazy cells contain abundant oxidative enzymes (86 151 171 205 206) In electronmicroscopical studies numerous mitochondria have been demonstrated in the oxyphilic cells in the parotid and parathyroid (171) and recently also in the thyroid (97) These morphological findings argue for a high metabolic activity which however does not appear to be involved in thyroglobulin synthesis They are thus invariably seen and often the dominating type of epithelial cell in the thyroid of patients with primary myxoedema (9 16 50, 117) or diffuse lymphoid thyroiditis with low thyroid uptake of ^{131}I and hypothyroidism (Fig 37, Chapter VII) After administration of ^{131}I the scintigram shows no uptake by non follicular Hurthle cell tumours (208) In autoradiographic studies after administration of ^{131}I to patients with diffuse lymphoid thyroiditis or Hurthle cell tumours little or no uptake were seen in the colloid in follicles lined by oxyphilic cells (63 137) and no uptake in the area with solid oxyphilic epithelial formations (63 66) It thus appears reasonable to suppose that the production of thyroid hormone will decrease with the conversion of epithelial cells to Askanazy cells which seems to be continuous in cases with diffuse lymphoid thyroiditis (Fig 19 Chapter IV) If the regeneration of the follicular epithelial cells is insufficient conversion of follicular epithelial cells to Askanazy cells is accompanied by hypofunction of the gland and the classical clinical picture of Hashimoto's disease occurs in middle age

During treatment with thyroid hormone the goitre regressed in 85 % of the patients with diffuse lymphoid thyroiditis a figure in agreement with that reported by previous workers in this field (see 7) The number of cases with regression of goitre was significantly*** higher in patients with diffuse

lymphoid thyroiditis than in those with colloid goitre (Table 21, Chapter VII). Doses of thyroid hormone exceeding 0.1 mg proved necessary to produce definite regression of the goitre (Table 22, Chapter VII).

The function and proliferation of thyroid epithelium is affected by thyroxin in a negative feedback-mechanism (see 42, 158). Various radioiodine studies of patients with diffuse lymphoid thyroiditis suggest an increased TSH production (see 183). In *in vitro* bio-assays, however, EL KABIR et al. (53) found decreased serum TSH values in certain forms of the disease. The therapeutic effect of thyroid hormone in diffuse lymphoid thyroiditis is believed to be due to the feedback mechanism, inhibiting production of TSH and thereby suppressing epithelial proliferation in the thyroid. It was shown (Chapter IV) that the ratio between follicular epithelial cells/1000 lymphoreticular cells and nucleated blood cells increases significantly** during thyroid hormone therapy and that this is probably due to a decrease in the amount of lymphoid tissue in the thyroid. This contrasts with the findings in laboratory animals which under certain experimental conditions showed hyperplasia of extrathyroid lymphoid tissue during treatment with thyroxin (81, 127).

Treatment with thyroid hormone did not appear to affect the incidence and titre of different thyroid antibodies. This is in accord with the findings of SKANSE & NILSSON (182), but is not consistent with those reported by OWEN & SMART (147) and LEBOEUF & DUCHARME (115).

Treatment with thyroid hormone is considered to be the therapy of choice in diffuse lymphoid thyroiditis (71, 134, 184, 185). It is therefore important to diagnose this type of thyroiditis without operation. As mentioned previously, it would appear that fine needle biopsy of the thyroid is at present the method of choice in the diagnosis of thyroiditis. Numerous reports suggest that even malignant thyroid processes (*e.g.* 19, 52, 67, 79, 126, 187, 198, 211) and also the development of a reticulum cell sarcoma in a previously known diffuse lymphoid thyroiditis (142) can be diagnosed by fine needle puncture biopsy. In the clinical treatment of lymphoid thyroiditis it is important, however, to stress that fine needle aspiration biopsy cannot exclude the possibility of a simultaneous malignant process of the thyroid in a patient with lymphoid thyroiditis. If the goitre does not regress after 2—3 months' treatment with adequate doses of thyroid hormone, which happens in cases of diffuse lymphoid thyroiditis (Chapter VII), surgical removal of thyroid tissue for histological examination is indicated.

CHAPTER IX

SUMMARY

The investigation was carried out on patients referred for fine needle aspiration biopsy of the thyroid as a link in an investigation of goitre and/or thyroid functional disorders.

1. The smears of fine needle aspirates from various types of thyroiditis showed the following cytological pictures:

In one case of acute suppurative thyroiditis studied the picture was characterised by protein rich fluid, necrotic material, numerous neutrophilic leucocytes and macrophages as well as intracellular bacteria.

In subacute thyroiditis (20 cases) the follicular epithelial cells contained increased numbers—and large aggregates—of paravacuolar granules and showed degenerative and proliferative changes. Inflammatory cells in the form of macrophages, mature lymphocytoid cells and neutrophilic leucocytes were seen. Multinucleated giant cells sometimes situated adjacent to colloid were also observed. The cytological picture varied markedly from one part of the goitre to another and from patient to patient. The numerous paravacuolar granules and the multinucleated giant cells were, however, constant characteristic findings.

In diffuse lymphoid thyroiditis (125 cases including 35 with histologically verified diagnosis) follicular epithelial cells were often seen in clusters and invariably showed proliferative changes. Pyknosis and karyorrhexis were rare. Askanazy cells and colloid were sometimes missing. Multinucleated giant cells were occasionally observed. Mature lymphocytoid cells usually dominated the smears and constituted 95 % of the lymphoreticular cells. Stem cells, mature tissue bound reticulum cells, plasma cells, monocytoid cells and large free phagocytes and mitoses constituted the rest of the lymphoreticular cells and occurred in decreasing number in the order given.

Of the above mentioned cytological findings, the occurrence of follicular epithelial polymorphism and a moderate to abundant amount of lymphoreticular cells are the cytological criteria for the diagnosis of diffuse lymphoid thyroiditis from fine needle aspiration smears.

A positive diagnosis of focal lymphoid thyroiditis was possible in 4 of the 17 cases studied. In these 4 cases lymphoreticular cells were seen in clusters or in only one of the aspirates obtained.

In the single case of Riedel's thyroiditis studied a few follicular epithelial cells not resembling Askanazy cells were seen as well as inflammatory cells in the form of mature lymphocytoid cells, neutrophilic leukocytes, plasma cells and macrophages. Fibroblasts and fibrocytes constituted 10 % of the nucleated cells.

2. The cytological picture of diffuse lymphoid thyroiditis varied with the patients' ages. Askanazy cells were missing or rare in children and adolescents. The relative number of Askanazy cells and plasma cells increased and the relative number of stem cells decreased with increasing age. The amount of colloid and the number of follicular epithelial cells/1000 lymphoreticular cells and nucleated blood cells did not vary significantly with age.

3. Thyroid puncture and cytological examination were repeated 4—44 months after the first puncture in 78 adult patients with diffuse lymphoid thyroiditis. The relative number of Askanazy cells was larger on the second occasion suggesting progressive conversion of the epithelial cells to Askanazy cells with the duration of thyroiditis or with the patient's age. In the patients treated with thyroid hormone there was a significantly** larger number of cases with an increased ratio between the number of follicular epithelial cells and lymphoreticular cells than with a decreased ratio. The increased ratio was probably due to repression of the lymphoid tissue in association with thyroid hormone treatment. No significant difference was found in the amount of colloid in the material aspirated on the two occasions.

4. In 6 patients with diffuse lymphoid thyroiditis partial or total thyroidectomy was performed and the cytological and histological findings were compared. Agreement was good regarding the amount of follicular epithelial cells, Askanazy cells, and lymphoreticular cells and fairly good regarding the amount of colloid and the degree of follicular epithelial polymorphism.

5. Adults (87) with cytologically diagnosed diffuse lymphoid thyroiditis, healthy subjects without thyroid disease, and patients with colloid goitre were examined for antibodies against thyroglobulin (TRC), cytoplasmic antigen (CFT + IFI), second colloid antigen (IFL) and thyroid cell surface antigen (MH). Antibodies against some thyroid antigen could be demonstrated in all patients with diffuse lymphoid thyroiditis but also in low titre in so many of the controls that only high titres *i.e.* serum dilution ≥ 1/1600 in the TRC and the MH tests, ≥ 1/10 in CFT and ≥ 1/25 in the IFL test were of diagnostic value in the diagnosis of diffuse lymphoid thyroiditis. Of the 87 patients with diffuse lymphoid thyroiditis 74 (85 %) had positive reactions at these levels in one or more antigen antibody tests.

In a comparison of the incidence and titre of various thyroid antibodies in 56 patients with diffuse lymphoid thyroiditis treated with thyroid hormone and 31 patients with diffuse lymphoid thyroiditis not treated with thyroid hormone and in 14 patients with diffuse lymphoid thyroiditis before and 4—18 months after the beginning of thyroid hormone

therapy no definite effect of treatment was demonstrable

The mean relative number of Askanazy cells was significantly* higher in the group with demonstrable antibodies against cytoplasmic antigen and second colloid antigen than in the group without the above mentioned antibodies No correlation was found between the relative number of plasma cells and the incidence and titre of the various thyroid antibodies

6 Diffuse lymphoid thyroiditis was diagnosed in 125 (14 %) of 894 patients examined with fine needle aspiration biopsy A negative significant** correlation was found between the relative number of Askanazy cells and the maximum thyroidal uptake of ^{131}I During treatment with thyroid hormone the goitre diminished in size in 53 (85 %) of 62 patients with diffuse lymphoid thyroiditis and in 44 % of the patients in an age and sex matched control series of non toxic colloid goitre L-thyroxin in a dose exceeding 0 1 mg/day had a better effect on the diminution in the size of the goitre than a smaller dose

Fig 39 *Hurthle cell adenoma The large Hurthle cells (Askanazy cells) are characterised by abundant cytoplasm often with well outlined cytoplasm and grey blue fine granules in cytoplasm (File No 422/64 MGG × 700)*

Fig 40 *Normal thyroid Follicular epithelial cells form a pseudosyncytium and their faintly staining cytoplasm contains paravacuolar granules (arrows) The round or slightly oval nuclei have no visible nucleoli Pale blue colloid to the right (File No 418/62 MGG × 700)*

Fig 41 *Acute suppurative thyroiditis Numerous partly devitalised neutrophilic leukocytes scattered in protein rich fluid Two cells (arrow) with intracellular bacteria (File No 274/65 MGG × 896)*

Fig 42 *Subacute thyroiditis Follicular cells with increased amount of paravacuolar granules which often aggregate within the enlarged vacuoles (File No 253/62 MGG, × 700)*

Fig 43 *Subacute thyroiditis Follicular cells with degenerated cytoplasm to left in illustration Cytoplasm is cloudily vacuolised and has distinct borders Paravacuolar granules in epithelial cells to right but not in the degenerated cells (File No 300/63 MGG × 700)*

Fig 44 *Subacute thyroiditis Follicular cells with severe degenerative changes in cytoplasm and with 3 pyknotic nuclei (arrow) (File No 34/61, MGG × 700)*

Fig 45 *Subacute thyroiditis in recovery phase Follicular epithelial cells show proliferative changes with different sized nuclei and with visible nucleoli in some nuclei Paravacuolar granules decreased in number and size (File No 687/64 MGG × 700)*

Fig 46 *Subacute thyroiditis Inflammatory cells in form of neutrophilic leukocytes (a) lymphocytes (b) and macrophages (c) (File No 207/64 MGG × 700)*

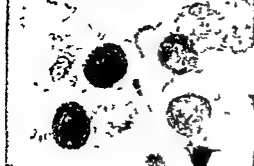


Fig 39



Fig 40

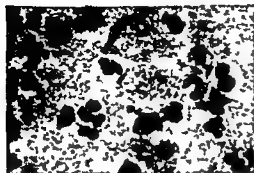


Fig 41

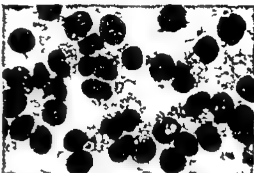


Fig 42

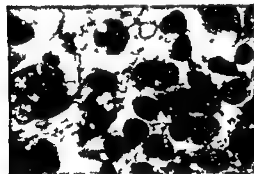


Fig 43



Fig 44

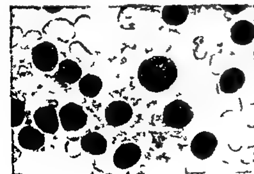


Fig 45



Fig 46

Fig 47 *Subacute thyroiditis Neutrophilic leukocytes and mononucleated inflammatory cells surrounding a macrophage containing abundant phagocytised material in the cytoplasm (File No 207/64 MGG × 700)*

Fig 48 *Subacute thyroiditis Uniform follicular epithelial cells with paravacuolar granules (right) and part of a multinucleated giant cell (left) (File No 346/62 MGG × 700)*

Fig 49 *Subacute thyroiditis Multinucleated giant cell containing paravacuolar granules (arrow) Two neutrophilic leukocytes at bottom (File No 248/64 MGG, × 700)*

Fig 50 *Diffuse lymphoid thyroiditis "Normal" follicular cells containing paravacuolar granules (File No 512/64 MGG × 700)*

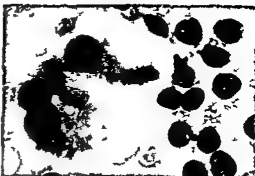
Fig 51 *Diffuse lymphoid thyroiditis Follicular epithelial cells surrounding colloid The size of the nuclei and range of variation of their size suggest proliferative changes (File No 360/64 MGG × 896)*

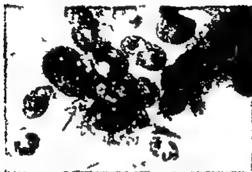
Fig 52 *Diffuse lymphoid thyroiditis Askanazy cells with typical granulations of cytoplasm (File No 722/64 MGG × 700)*

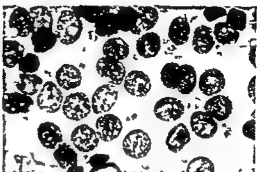
Fig 53 *Diffuse lymphoid thyroiditis Polymorphous picture of Askanazy cells varying widely in size (File No 695/64 MGG × 700)*

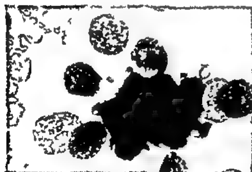
Fig 54 *Diffuse lymphoid thyroiditis Follicular epithelial cells of Askanazy type to left and with foamy vacuolised cytoplasm to right (File No 101/63 MGG × 700)*

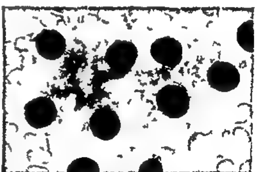

Fig 47


Fig 48


Fig 49


Fig 50


Fig 51


Fig 52

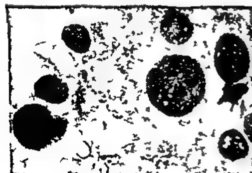

Fig 53


Fig 54

Fig 55 *Diffuse lymphoid thyroiditis Askanazy cells with pyknosis and karyorrhexis (arrow)* (*File No 118/62 MGG × 896*)

Fig 56 *Diffuse lymphoid thyroiditis The picture is dominated by mature lymphocytoid cells* (*File No 40-/62 MGG × 700*)

Fig 57 *Diffuse lymphoid thyroiditis Mature lymphocytoid cells a plasma cell (a) and a mature tissue bound reticulum cell (b) characterised by an oval nucleus loose chromatin network and absence of visible outline of cytoplasm* (*File No 743/64 MGG × 700*)

Fig 58 *Diffuse lymphoid thyroiditis Mature lymphocytoid cells and numerous small stem cells with narrow basophilic cytoplasm (Soderstrom's NBC cell 198)* (*File No 413/62 MGG × 700*)

Fig 59 *Diffuse lymphoid thyroiditis Mature lymphocytoid cells non phagocytising mature tissue bound reticulum cell (a) and large stem cell (b) with strongly basophilic cytoplasm and perinuclear pale area* (*File No 413/6- MGG × 896*)

Fig 60 *Case 1 (A P File No 600/64) Cytological diagnosis Well differentiated thyroid carcinoma Colloid surrounded by follicular epithelial cells with nuclei varying in stainability size and shape* (*MGG × 700*)

Fig 61 *Same specimen as in Fig 60 Follicular epithelial cells with distinct nucleoli Note the large nucleolus at arrow* (*MGG × 700*)

Fig 6- *Case 2 (E O File No 720/63) Cytological diagnosis Diffuse lymphoid thyroiditis with atypical lymphocytoid cells The reproduction shows atypical lymphocytoid cells with small vacuoles in the cytoplasm and with folded different shaped nuclei Observe difference in appearance of these lymphocytoid cells compared with that of corresponding cells in Fig 56* (*MGG × 700*)



Fig 55

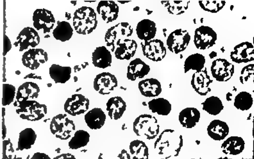


Fig 56

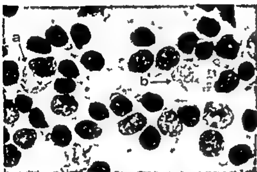


Fig 57

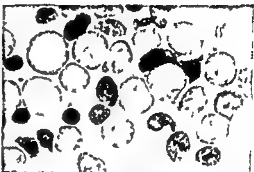


Fig 58



Fig 59

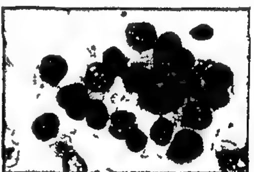


Fig 60

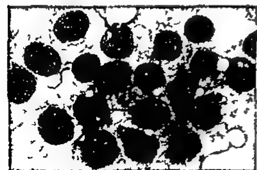


Fig 61

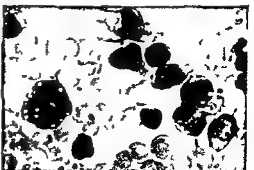


Fig 62

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REFERENCES

1 Abramov M G Klinitcheskaja Zitologia Medgiz Moscow 196-

2 Aho K Harboe, M & Leikola J Studies on the antibody nature of the rheumatoid factor with sheep erythrocytes sensitized with human anti sheep cell antibodies and with ORh positive cells with incomplete anti Rh antibodies Immunology 7 403 1964

3 Anderson J W McConahey W M Alarcon Segovia D Emslander R F & Wakim K G Diagnostic value of thyroid antibodies J Clin Endocr 27 937 1967

4 Anderson W A D Pathology Mosby Saint Louis 1966 p 1096

5 Andre R & Dreyfus B Sur les tumeurs thyroidiennes latero-cervicales dites aberrantes L aide apportee au diagnostic par la ponction Presse Med 64 330 1956

6 Askanazy M Pathologisch-anatomische Beitrage zur Kenntniss des Morbus Basedowii insbesondere uber die dabei auftretende Muskelerkrankung Deutsch Arch Klin Med 61 118 1898

7 Astwood E B Cassidy C E & Auerbach G D Treatment of goiter and thyroid nodules with thyroid J A M A 174 459 1960

8 Balfour B M Doniach D Roitt I M & Couchman K G Fluorescent antibody studies in human thyroiditis Auto-antibodies to an antigen of the thyroid colloid distinct from thyroglobulin Brit J Exp Path 42 307 1961

9 Bastenie M P A Contribution a l etiologie du myxoedeme spontane de l adulte Bull Acad Roy Med Belg 9 179 1944

10 Bastenie P A Neve, P Bonnyns M Ermans A M & Malaisse Lagae, F Thyroidite chronique latente et myxoedeme spontane de l adulte Ibid 7 139 1965

11 Bastenie, P A Neve, P Bonnyns M & Vanhaelst L Clinical and pathological significance of asymptomatic atrophic thyroiditis A condition of latent hypothyroidism Lancet 1 915 1967

12 Beahrs O H Woolner L B Engel S & McConahey W M Needle biopsy of the thyroid gland and management of lymphocytic thyroiditis Surg Gynec Obstet 114 636 1962

13 Beall G N & Solomon D H Inhibition of long-acting thyroid stimulator by thyroid particulate fractions J Clin Invest 45 552 1966

14 Becker K L Titus J L Woolner L B & McConahey W M Significance of morphologic thyroiditis Ann Intern Med 62 1134 1965

15 Belyavin G & Trotter W R Investigations of thyroid antigens reacting with Hashimoto sera Lancet 1 645 1959

16 Berkheisser S W Adult hypothyroidism Report of an advanced case with autopsy study J Clin Endocr 15 44 1955

17 Bessis M C & Breton Gorius J Iron metabol sm in the bone marrow as seen by electron m croscopy A cr t cal rev ew Blood 19 635 1962

18 Bleha O Engelberth O Jezková Z & Sramková J Ant bod es in various clin cal cond ons of the thyro d Rev Czech Med 11 246 1965

19 Bonneau H Sommer D & de The G Le cyto-diagnostic dans les tumeurs thyro diennes Sud Med Ch r 8142 1959

20 Bonneau H Sommer D & de T ie G Étude cr t que de la cytolog e tumorale par ponct on a l a gu lle f ne a propos de 905 observat ons Presse Med 68 909 1960

21 Boyd W A text book of pathology Kimpton London 1947 p 771

2 Boyden S V The adsorpt on of prote ns on erythrocytes treated with tann c acid and subsequent hemagglut nat on by antiprote n sera J Exp Med 93 107 1951

23 Buchanan W W Alexander W D Crooks J Koutras D A Wayne E J Anderson J R & Gold e R B Associat on of thyrotoxicos s and autoimmune thyro d t s Br t Med J 1 843 1961

4 Buchanan W W & Harden R McG Pr mary hypothyro d sm and Hashimoto s thyro d tis Arch In ern Med 115 411 1965

25 Buchanan W W Harden R McG & Clark, D H Hash moto s thyro d t s Its d fferentiat on from s mple go tre thyro d neoplasm drug nduced go tre and dyshormonogenes s Brit J Surg 52 430 1965

6 Buchanan W W Koutras D A Crooks J Alexander W D Brass W Anderson J R Goudie, R B & Gray K G The cl n cal s gn f cance of the complement fixat on test in thyrotox cos s J Endocr 24 115 1962

27 Cervino J M Paseyro P Grosso O F & Estable M F El citograma t ro deo l nfo de con presenc a de ant cuerpos ant t ro deos en el suero sangu neo An Fac Med Montev 44 230 1959

28 Cervino J M Paseyro P Grosso O F & Maggiolo J La exploracion c tolog ca de la glandula tiro des y sus correlac ones anatomocl n cas Ib d 47 128 196

29 Chesky V E Dreese W C & Hellwig C A Chron c thyro d t s supravital stud es of surg cal go er specimens Surg Gynec Obstet 93 575 1951

30 Chesky V E Hellwig C A & Welch J W Cancer of the thyro d assoc ated w th Hash moto s d sease an analys s of forty-e ght cases Amer Surg 28 678 1962

31 Choisette G Auriol M Brochériou C & Pinaudeau Y La thyro d te lymphocyta re latente chez le v e llard Étude morpholog que et s gn f cat on Ann Anat Path (Par s) 11 55 1966

32 Clausen H J Exper mental product on of struma lymphomatosa Proc Soc Exp B ol Med 83 835 1953

33 Clayton G W & Johnson C M Struma lymphomatosa in ch ldren J Ped at 57 410 1960

34 Cline M J Selenkow H A & Brooke M S Auto mmun ty n thyro d d sease New Eng J Med 260 117 1959

35 Crile Jr G Thyro d t s Ann Surg 127 640 1948

36 Crile Jr G Thyro d t s Ann Intern Med 37 519 1952

37 Crile Jr G & Hazard J B Class f ca t on of thyro d t s w th spec al reference to the use of needle b opsy J Cl n Endocr 11 1123 1951

38 Crile Jr G & Hazard J B Incidence of cancer n struma lymphoma osa Surg Gynec Obstet 115 101 1962

39 Dailey M F Lindsay S & Skahen R Relat on of thyro d ne plasms to Hashi moto d sease of the thyro d gland A M A Arch Surg 70 291 1955

40 Decourt J Eyquem A Savoie J & Calmettes C Les phenomènes d auto immun sat on en patholog e thyro d enne Sem Hop Par s 40 1851 1964

41 Derrien Y Michel R & Roche J Recherches sur la préparat on et les propr etés de la thyroglobul ne pure B och m B ophys Acta 2 454 1948

42 Dobyns B M The thyro d p tu tary relat onsh ps In The thyro d (eds Hazard J B & Smith D E) p 76 W ll ams & W lk ns Balt more 1964

43 Doniach D Thyro d auto mmune d s ease In Sympos um on the thyro d gland (eds McGo an G K & Sandler M) J Cl n Path Suppl 20 385 1967

44 Doniach D & Hudson R V Lymph adeno d go tre (Hash moto s d sease) Diagnost c and b ochem cal aspects Brit Med J 1 672 1957

45 Doniach D Hudson R V & Roitt I M Human auto- mmune thyro d tis Cl n cal stud es Ib d 1 365 1960

46 Doniach D Nilsson L R & Roitt I M Auto mmune thyro d t s n ch l lren and adolescents II Immunolog cal c r relations and parent study Acta Paed at Scand 54 260 1965

47 Doniach D & Roitt I M Auto mmunity in Hash moto s d sease and s impl cat ons J Cl n Endocr 17 1293 1957

48 Doniach D & Roitt I M Present concepts of thyro d auto-immunity In Modern trends n endocrinology (ed Gard ner H ll H) p 278 Butterw rth London 1961

49 Doniach D & Roitt I M Cl n cal appl cat n f thyr l auto-ant body tests In Cl n cal a pects f mmunology (eds Cell P G H & Coombs R R A) p 611 Blackwell Oxf rd 1962

50 Douglass P [illegible] S D Path [illegible] Survey [illegible] 17 1354 [illegible]

51 Dvorak V Cross K & Stara B Asp rat on punct on cyt l gy n nc log cal pract ce Acta un nt cancer 20 807 1964

52 Einhorn J & Franzen S Th n needle b opsy in the d agn s s of thyr d d sease Acta Rad l (Stockh lm) 58 321 1962

53 El Kabir D J Doniach D & T rner Warwick R Serum content of thyr tropi c h rmone in human thyr i t s Low TSH levels w th h gh rad d ne uptake in m ld aut mmune thyro d tis J Cl n Endocr 23 510 1963

54 Ernstr m U Thyrox n induced changes in cell composit on of lymph n de t ssue spleen and thymus Acta Path M cr b l Scand 59 145 1963

55 Espmark J Å & Fagraeus A Detec t on of ant gens in t ssue culture with the a d f mixed hemadsorpt n Ib d Suppl 154 258 1962

56 Espmark J Å & Fagraeus A I lent f cat on of the spec es [illegible] cells by m xed hemads rpt n A m xe l a t globul n react on appl ed t m n l yer cell cultures J Immun 94 530 1965

57 Fagraeus A Ant b dy pr duct n i relat n to the devel pment f plasma cells In viv and in v tr exp r m ts Acta Me l Scan l Suppl 204 1948

58 Fagraeus A N menclature f im [illegible] l g cally c mpetent cells [illegible] Sympos um [illegible] Cell lar aspects f immun ty (eds W lste [illegible] & O C nn r M) [illegible] London 19[illegible]

59 Fagraeus A & Espmark Å [illegible] mixe l [illegible] (London) [illegible] 37 [illegible]

60 Fagraeus A Espmark J Å [illegible]

61 Fahey J L & Goodman H C Characterization of anti-thyroglobulin factors in human serum J Clin Invest 39 1-39 1960

62 Fetisov A U Cytological diagnosis of diseases of the thyroid gland Lab Delo 8 22 1962

63 Fitzgerald P J Foote, F W & Hill R F Concentration of I^{131} in thyroid cancer shown by radioautography Cancer 3 86 1950

64 Forbes I J Roitt I M Doniach D & Solomon I L The thyroid cytotoxic autoantibody J Clin Invest 41 996 1962

65 Foster G V MacIntyre I & Pearse A G E Secretion of calcitonin by the mitochondrion rich cells of the thyroid In Current topics in thyroid research (eds Cassano C & Andreoli M) p 221 Acad Press New York 1965

66 Frantz V K Quimby E H & Evans T C Radioactive iodine studies of functional thyroid carcinoma Radiology 51 532 1948

67 Frazell E L & Foote Jr F W Papillary cancer of the thyroid Cancer 11 895 1958

68 Frid G & Wijnbladh Hj Subacute thyroiditis struma lymphomatosa (Hashimoto's disease) and chronic fibrous invasive goitre (Riedel's disease) Acta Chir Scand 112 170 1956

69 Friedman N B Cellular involution in the thyroid gland Significance of Hurthle cells in myxedema exhaustion atrophy Hashimoto's disease and the reactions to irradiation thiouracil therapy and subtotal resection J Clin Endocr 9 874 1949

70 Fulthorpe A J Roitt I M Doniach D & Couchman K A stable sheep cell preparation for detecting thyroglobulin auto-antibodies and its clinical applications J Clin Path 14 654 1961

71 Furr Jr W E & Crile Jr G Struma lymphomatosa Clinical manifestations and response to therapy J Clin Endocr 14 79 1954

72 Glynn L E & Holborow E J Autoimmunity and disease Blackwell Oxford 1965

73 Godwin J T Aspiration biopsy Technique and application Ann NY Acad Sci 63 1348 1956

74 Godwin J T Aspiration biopsy J Med Ass Georgia 46 537 1957

75 Goetsch E & Kamner M Chronic thyroiditis and Riedel's struma etiology and pathogenesis The role of hyperfunctioning parenchyma of primary hyperplastic goiter J Clin Endocr 15 1010 1955

76 Goudie R B Anderson J R & Gray K G Complement fixing antithyroid antibodies in hospital patients with asymptomatic thyroid lesions J Path Bact 77 389 1959

77 Graham R The significance of lymphadenoid changes in the thyroid gland J Endocr 7 1 1950

78 Greer M A & Astwood E B Treatment of simple goiter with thyroid J Clin Endocr 13 1312 1953

79 Grosso O F Pastyro P & Maggiolo J Contribucion al diagnostico del cancer del tiroides A proposito de 40 observaciones de carcinoma infiltrativo An Fac Med Montev 44 53 1959

80 Guthrie C G Gland puncture as a diagnostic measure Bull Hopkins Hosp 32 266 1921

81 Gyllensten L Influence of the thymus and thyroid on the postnatal growth of the lymphatic system in guinea pigs Acta Anat (Basel) Suppl 18 1953

82 Hackett E Beech M & Forbes I J Thyroglobulin antibodies in patients without clinical disease of the thyroid gland Lancet 2 402 1960

83 Hahn Jr H B Hayles A B & Woolner L B Lymphocytic thyroiditis in children J Pediat 66 73 1965

84 Halberg P Thyro-cytotoxic factor Acta Med Scand 177 309 1965

85 HALBERG P & WERDELIN O Kronisk thyreoiditis — Kliniske fund hos 35 histologisk verificerede tilfælde Ugeskr Læg 127 1283 1965

86 HALEY H L DEWS G M & SOMMERS S C A histochemical comparison of primary thyroid hyperplasia and adenomatous goiter A M A Arch Path 59 635 1955

87 HALL R Immunologic aspects of thyroid function New Eng J Med 266 1204 1962

88 HAM A W Histology Lippincott Philadelphia 1957 p 712

89 HAMLIN JR E & VICKERY A L Needle biopsy of the thyroid gland New Eng J Med 254 742 1956

90 HAMPERL H Onkocytes and the so-called Hurthle cell tumor A M A Arch Path 49 563 1950

91 HASHIMOTO H Zur Kenntniss der lymphomatosen Veranderung der Schilddruse (Struma lymphomatosa) Arch Klin Chir 97 219 1912

92 HAZARD J B Thyroiditis A review Part I Amer J Clin Path 25 289 1955

93 HAZARD J B Thyroiditis A review Part II Ibid 25 399 1955

94 HAZARD J B & SENHAUSER D A Recent studies in thyroiditis Immunopathological correlations In Evaluation of thyroid and parathyroid functions (eds Sunderman F W & Sunderman Jr F W) p 118 Lippincott Philadelphia 1963

95 HAWK W A CRILE JR G HAZARD J B & BARRETT D L Needle biopsy of the thyroid gland Surg Gynec Obstet 122 1053 1966

96 HEIMANN P Ultrastructure of human thyroid A study of normal thyroid untreated and treated diffuse toxic goiter Acta Endocr (Kobenhavn) Suppl 110 1966

97 HEIMANN P Ultrastructure of human thyroid in thyrotoxicosis In Thyrotoxicosis (ed Irvine W J) p 155 Livingstone Edinburgh 1967

98 HEIMANN P & SCHNÜRER L B Needle biopsy of the thyroid gland A report of 117 cases with special reference to the diagnostic accuracy of the method in benign thyroid disorders Acta Chir Scand 128 85 1964

99 HELLWIG C A Histology of thyroid in exophtalmic goiter and hyperthyroidism Arch Path (Chicago) 28 870 1939

100 HIRABAYASHI R N & LINDSAY S The relation of thyroid carcinoma and chronic thyroiditis Surg Gynec Obstet 121 243 1965

101 HJORT T Autoimmunisering ved thyreoidea lidelser Metodologiske kliniske og teoretiske problemer Aarhus Stiftsbog trykkerie 1963

102 HOLBOROW E J BROWN P C ROITT I M & DONIACH D Cytoplasmic localization of complement fixing autoantigen in human thyroid epithelium Brit J Exp Path 40 583 1959

103 HURTHLE K Beitrage zur Kenntniss des Secretionsvorgangs in der Schilddruse Arch Physiol 56 1 1894

104 IRVINE W J Studies on the cytotoxic factor in thyroid disease Brit Med J 1 1444 1962

105 JAFFIOL C PAGES A & SAADE F Anticorps antithyroidens et maladies thyroidiennes Rev Franc Endocr Clin 5 393 1964

106 JENNINGS J F & TAYLOR G Studies on the human thyroid epithelial cell antigen Brit J Exp Path 47 201 1966

107 JOLL C A The pathology diagnosis and treatment of Hashimoto s disease (struma lymphomatosa) Brit J Surg 27 351 1939

108 JONSSON J FAGRAEUS A & BIBERFELD G The mixed haemadsorption test as an aid in the diagnosis of thyroid autoimmune diseases To be published

109 JONSSON J FAGRAEUS A & ESPMARK J Å Auto-antibodies to thyroid antigens studied by the mixed haemadsorption technique In Proc 10th congr int blood transf Stockholm 1964 p 79 Karger Basel 1965

110 Kabat E. A & Mayer M M Experimental immunochemistry Thomas Springfield Ill. 1961 p 299

111 Kohler P O Floyd W L & Wynn J Indications for thyroid needle biopsy Suppurative thyroiditis Southern Med J 59 18. 1966

112 Lamberg B-A Hernberg C. A. & Hakkila R Treatment of non toxic goitre with thyroid preparations Acta endocr 33 384 1960

113 Leboeuf G & Bongiovanni A M Thyroiditis in childhood In Advances in pediatrics Vol. XIII (ed. Levine S S) p 183 Yr Bk. Pub Chicago 1964

114 Leboeuf G Bongiovanni A. M Striker D D & Eberlein W R Immunologic and thyroid function studies in euthyroid children with goiter J Pediat 58 477 1961

115 Leboeuf G & Ducharme, J R Thyroiditis in children diagnosis and management Pediat. Clin. N Amer 13 29 1966

116 Lennert K Handbuch der speziellen pathologischen Anatomie und Histologie Bd. I 3 A Lymphknoten Diagnostik in Schnitt und Ausstrich. Springer Berlin 1961

117 Lennox B The large-cell small acinar thyroid tumour of Langhans and the incidence of related cell groups in the human thyroid J Path Bact 60 295 1948

118 Leonhardt T Thyreoidit. Lakartidningen 1 914 1965

119 Levitt T Evolution of the toxic thyroid gland A clinical and pathological study based on 2114 thyroidectomies Lancet 261 957 1951

120 Lewontin R C. & Felsenstein J The robustness of homogeneity tests in 2 × N tables Biometrics 21 19 1965

121 Lindsay S Carcinoma of the thyroid gland Thomas Springfield Ill 1960

[illegible] S Pathology of the thyroid The thyroid gland (eds Pitt Rivers R & R) Vol. 2 London 1964

123 Lindsay S & Dailey M E. Granulomatous or giant cell thyroiditis A clinical and pathologic study of 37 patients Surg. Gynec Obstet 98 197 1954

124 Lindsay S Dailey M Friedlander J Yee C & Soley M Chronic thyroiditis A clinical and pathologic study of 354 patients J Clin. Endocr 12 1578 1952

125 Lopes Cardozo P Clinical cytology Stafleu Leyden 1954

126 Lopes Cardozo P Le cytodiagnostic immediat par la ponction thyroidienne Arch Anat Path (Paris) 12 23 1964

127 Lundin P M Anterior pituitary gland and lymphoid tissue growth Acta Endocr Suppl 40 1958

128 Mackay I R & Perry B T Autoimmunity in human thyroid disease Aust Ann Med 9 84 1960

129 Martin H E. & Ellis E B Biopsy by needle puncture and aspiration Ann Surg 92 169 1930

130 Masi A T Hashimoto s disease An epidemiological study based on a community wide hospital survey J Chronic Dis 18 35 1965

131 Masi A T Hartmann W H & Shulman L E. Hashimoto s disease An epidemiological critique Ibid 18 1 1965

132 Masi A T Sartwell P E Hartmann W H & Shulman L E Hashimoto s disease A study of the consistency of its histopathological diagnosis Ibid 18 23 1965

133 May F Netter A & Bloch Michel H La ponction du corps thyroide Ann Endocr (Paris) 12 632 1951

134 McConahey W M Woolner L B Black B M & Keating Jr F R Effect of desiccated thyroid in lymphocytic (Hashimoto s) thyroiditis J Clin Endocr 19 45 1959

135 McKenzie J M Review Pathogenesis of Graves s disease Role of the long acting thyroid stimulator J Clin Endocr 25 424 1965

136 MENEGAUX G NETTER A & YANEVA H Cancer thyro d en d agno t que par l examen cytolog que du frot s thyro d en Bull Soc Med Hop Par s 67 55 1951

37 MEYER J S An autoradiograph c t me study of prote n bound odine in the human thyro d gland Endocr nology 70 278 1962

138 MOE CHLIN S D e M lzpunk on Schwa be Basel 1947

139 MORTENSEN J D WOOLNER L B & BENNET W A Gross and m croscop c f nd ngs n clinically normal thyro d glands J Clin Endocr 15 1270 1955

140 MUNRO D S Aet ology of hyper thyro d sm In Sympos um on the thyro d gland (ed McGowan G K & Sandler M) J Clin Path Suppl 20 368 967

141 MYREN J & S ERTS EN E Thin needle b opsy of the thy ro d gland n the d agnos of thyrotox cos s Acta Endocr 39 431 196

14 NIKIT NA N I Cytod agnos s of Hash mo s lymphomatous go tre Vop Onkol 11 36 1965

143 N LSSON L R & DON ACH D Auto-mmune thyro dit s n ch ldren and adolescen s I Cl nical stud es Acta Paed at Scand 53 55 1964

144 N LSSON L R & PERSSON P S Cyto-log cal asp ra on b opsy *adolescent* go tre Ib d 53 333 964

145 NOSSAL G J V & M TC ELL J The thymus n rela on to mmunolog cal tolerance In C ba Founda on Sympos um on Thymus Exper mental and clin cal stud es (ed Wolstenholme G E W & Porter R) p 105 Church ll London 1966

146 O GOOD E E & SE AN A J The cellular compos on of normal bone marrow as obtained by s ernal punc ure Phys ol Re 24 46 944

47 OWEN S G & S ART G A Thyro d an bod es n *myxoedema* Lancet 2 1034 958

148 PAINE J R TERPLAN K ROSE, N R WITEBSKY E & EGAN R W A clinical study of chron nonInfectious thyro ditis and auto mmunizat on Surgery 42 799 957

149 PASETRO P & GROSSO O F El cito-grama iro deo obten do por puncion sus apl caciones clin cas Med Panamer 6 15 1956

150 PAVLOVSKY A La punción ganglionar Lopez Buenos Aires 1934

151 PEARSE A G E The cytochemistry of the thyro d C cells and the r relationship to calc tonin Proc Roy Soc (B ol) 164 478 1966

5 PERSSON P S Cytolog c diagnos s of subacu e and chronic thyro d t s Acta Path M crob ol Scand 58 395 1963

153 PERSSON P S Unpublished observa t ons

54 P LP J R WE R D M STUART A E & IR NE W J A latex part cle precip ta tion test n the diagnos s of thyro d diseas J Clin Path 15 148 1962

155 P AGG O-BLANCO R A PASEYRO P & GROSSO O F El c ograma t ro deo Su n eres clinico Arch Urug Med C r Esp 32 81 1948

156 PORTER D D & FENELL R H Serologic and mmunoh stochemical study of human and exper mental thyro ditis New Eng J Med 265 830 1961

157 PUL ERTAFT R J V DONIAC D ROITT I M & HUDSON R V Cyto ox c effec s of *Hash* mo o serum on human thyro d cells in t ssue culture Lancet 2 2 4 1959

158 PURVES H D *Control of thyro d funct on* In The thyro d gland (eds P t R vers R & TROTTER W R) Vol 2 p 1 Butter worth London 1964

159 DE QUER A N F D e aku e nicht e terige Thyreo d s und d e Beteiligung der Schilddruse an aku en In ox kat onen uberhaupt M tt Grenzgeb Med Chir (Jena) Suppl 1904

160 RANSTROM S Malignant lymphoma of the thyroid and its relation to Hashimoto s and Brill Symmers disease Acta Chir Scand 113 185 1957

161 RAWSTRON J R & FARTHING C P A comparison of tests for thyroglobulin antibody J Clin Path 15 153 1962

162 RIEDEL B M C L Die chronische zur Bildung eisenharter Tumoren fuhrende Entzündung der Schilddrüse Verh Deutsch Ges Chir 25 101 1896

163 RIGGS J I SEWALD R J BURCKHALTER J H DOWNS C M & METCALF T G Isothiocyanate compounds as fluorescent labeling agents for immune serum Amer J Path 34 1081 1958

164 ROBERTS L Primary reticulosarcoma of the thyroid gland Postgrad Med J 37 481 1961

165 ROHR K Das menschliche Knochenmark Thieme Stuttgart 1960

166 ROITT I M & DONIACH D Human auto-immune thyroiditis serological studies Lancet 2 1027 1958

167 ROITT I M & DONIACH D Thyroid auto-immunity Brit Med Bull 16 152 1960

168 ROITT I M DONIACH D CAMPBELL P N & HUDSON R V Auto-antibodies in Hashimoto s disease (lymphadenoid goitre) Lancet 2 820 1956

169 ROSE N R & WITEBSKY E Studies on organ specificity V Changes in thyroid glands of rabbits following active immunization with rabbit thyroid extracts J Immun 76 417 1956

170 ROSE N R WITEBSKY E & BEIERWALTES W H Thyroiditis In Immunological diseases (ed Samter M) p 821 Little Boston 1965

171 ROTH S I OLEN E & HANSEN L S The eosinophilic cells of the parathyroid (oxyphil cells) salivary (oncocytes) and thyroid (Hurthle cells) glands Light and electron microscopic observations Lab Invest 11 933 1962

172 RUDOWSKI W Critical evaluation of aspiration biopsy in the diagnosis of tumours of the thyroid Amer J Surg 95 40 1958

173 SAXENA K M & CRAWFORD J D Juvenile lymphocytic thyroiditis Pediatrics 30 917 1962

174 SCHADE R O K OWEN S G SMART G A & HALL R The relation of thyroid auto-immunity to round-celled infiltration of the thyroid gland J Clin Path 13 499 1960

175 SCHLEUSENER H SCHULTZ R & GRUNER H Die Zytodiagnostik kalter Schilddrüsenknoten Med Klin 60 709 1965

176 SCHMIDT H J HENNING N & WITTE S Autoradiographische Untersuchungen des menschlichen intrathyreoidalen Jodstoffwechsels Deutsch Arch Klin Med 208 505 1963

177 SEGERDAHL E Über Sternalpunktionen Acta Med Scand Suppl 64 1935

178 SENHAUSER D A Immunopathologic correlations in thyroid diseases In The thyroid (eds Hazard J B & Smith D E) p 167 Williams & Wilkins Baltimore 1964

179 SENHAUSER D A HAZARD J B WILLIAMS E C & CRILE JR G The incidence of circulating thyroid antibodies as measured by means of two agglutination technics Amer J Clin Path 38 482 1962

180 SEVER J L Application of a microtechnique to viral serological investigations J Immun 88 320 1962

181 SIMMONDS M Über lymphatische Herde in der Schilddruse Virchow Arch Path Anat 211 73 1913

182 SKANSE B & NILSSON S B Thyroid antibodies in hypothyroidism Acta Med Scand 170 461 1961

183 SKILLERN P G Thyroiditis In The thyroid gland (eds Pitt Rivers R & TROTTER W R) Vol 2 p 130 Butterworth London 1964

184 SKILLERN P G Treatment of Hashimoto s disease and invasive thyroiditis In Modern treatment (eds Becker E L & Carr Jr E A) p 159 Harper & Row New York 1964

185 SKILLERN P G CRILE JR G McCULLAGH E P HAZARD J B LEWIS L A & BROWN H Struma lymphomatosa Primary thyroid failure with compensatory thyroid enlargement J Clin Endocr 16 35 1956

186 SKILLERN P G NELSON H E & CRILE JR G Some new observations on subacute thyroiditis J Clin Endocr 16 1422 1956

187 ŠKRABALO Z ČREPINKO I Grgic Z & HALPTMAN E Primjena aspiracione citodijagnostike kod bolesti stitnjace Lijecn Vjesn 83 1035 1961

188 SMART G A & OWEN S G Thyroid antibodies and thyroiditis Postgrad Med J 36 442 1960

189 SMART G A & OWEN S G Thyroiditis and hypothyroidism J Chronic Dis 14 537 1961

190 SNEDECOR G W Statistical methods Iowa State University Press Iowa 1956

191 STAHEL R Differentialdiagnose der Halscysten durch die Punktionsmethode Deutsch Arch Klin Med 189 3-9 1942

19 STAVITSKY A B Haemagglutination and haemagglutinationinhibition reactions with tannic acid and bis diazotized benzidin protein conjugated erythrocytes In Immunological methods (ed Ackroyd J F) p 368 Blackwell Oxford 1964

193 STEWART F W The diagnosis of tumors by aspiration Amer J Path 9 801 1933

194 STREICHNER H & SANDKUHLER S Klinische Zytologie Thieme Stuttgart 1953

195 STRUNGE T La ponction des ganglions lymphatiques Munksgaard Kobenhavn 1944

196 SODERSTROM N Puncture of goiters for aspiration biopsy Acta Med Scand 144 237 1952

197 SODERSTROM N Identification of normal tissues and tumors by cytologic aspiration biopsy Acta Soc Med Upsal 63 53 1958

198 SODERSTROM N Fine needle aspiration biopsy Used as a direct adjunct in clinical diagnostic work Almqvist Stockholm 1966

199 TAKATZY G The use of spiral loops in serological and virological micro-methods Acta Microbiol Acad Sci Hung 3 191 1955

200 TAYLOR S Subacute thyroiditis Postgrad Med J 33 327 1957

201 TEMPKA T ALEKSANDROWICZ J & TILL M Le thyroidogramme Sang 19 336 1948

202 TEMPKA T CEMBALA D KOWALCZYK M & URASIŃSKI I The thyroidgram Acta Med Pol 1 111 1960

203 THOMAS JR W C ANDERSON R M JURKIEWICZ M J ARAUJO J D & BLIZZARD R M Clinical studies in thyroiditis Ann Intern Med 63 808 1965

204 TORRIGIANI G & ROITT I M Sedimentation characteristics of human thyroid autoantibodies Immunology 6 73, 1963

205 Tremblay G Histochemical study of cytochrome oxidase and adenosine triphosphatase in Askanazy cells (Hurthle cells) of the human thyroid Lab Invest 11 514 1962

206 TREMBLAY G & PEARSE A G E Histochemistry of oxidative enzyme systems in the human thyroid with special reference to Askanazy cells J Path Bact 80 353 1960

207 TROTTER W R BELYAVIN G & WADDAMS A Precipitating and complement fixing antibodies in Hashimoto s disease Proc Roy Soc Med 50 961 1957

208 VALENTA L & JIRASEK J E Les carcinomes trabeculaires de la glande thyroide Rev Franc Endocr Clin 7 227 1966

209 Vickery A L & Hamlin Jr E Struma lymphomatosa (Hashimoto s thyroiditis) Observations on repeated biopsies in sixteen patients New Eng J Med 264 226 1961

210 Volpe, R & Johnston McA W Subacute thyroiditis a disease commonly mistaken for pharyngitis Canad Med Ass J 77 297 1957

211 Watson W L & Pool J L Cancer of the thyroid Surg Gynec Obstet 70 1037 1940

212 Weaver D R Deodhar S D & Hazard J B A characterization of focal lymphocytic thyroiditis Cleveland Clin Quart 33 59 1966

213 Welch J W & Hellwig C A Needle biopsy of the thyroid gland An evaluation J Int Coll Surg 40 483 1963

214 Weller T H & Coons A H Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro Proc Soc Exp Biol Med 86 789 1954

215 Werdelin O Thyreoidealidelsernes histologi Nord Med 78 879 1967

216 Werner S C The thyroid Hoeber New York 1955 p 756

217 Wetzel B K Spicer S S & Wollman S H Changes in fine structure and acid phosphatase localization in rat thyroid cells following thyrotropin administration J Cell Biol 25 593 1965

218 Whitesell Jr F B & Black B M A statistical study of the clinical significance of lymphocytic and fibrocytic replacements in the hyperplastic thyroid gland J Clin Endocr 9 1202 1949

219 Williams G M Ontogeny of the immune response II Correlations between the development of the afferent and efferent limbs J Exp Med 124 57 1966

220 Williams E D & Doniach I The post mortem incidence of focal thyroiditis J Path Bact 83 255 1962

221 Williams G M & Nossal G J V Ontogeny of the immune response I The development of the follicular antigen trapping mechanism J Exp Med 124 47 1966

222 Winter J Eberlein W R & Bongiovanni A M The relationship of juvenile hypothyroidism to chronic lymphocytic thyroiditis J Pediat 69 709 1966

223 Witebsky E & Rose N R Studies on organ specificity IV Production of rabbit thyroid antibodies in rabbit J Immun 76 408 1956

224 Witebsky E & Rose N R Autoimmunity and its relationship to thyroid diseases New York J Med 63 56 1963

225 Witebsky E Rose N R Terplan K Paine J R & Egan R W Chronic thyroiditis and autoimmunization J A M A 164 1439 1957

226 Woolner L B Thyroiditis Classification and clinicopathologic correlation In The thyroid (eds Hazard J B & Smith D E) p 123 Williams & Wilkins Baltimore 1964

227 Woolner L B McConahey W M & Beahrs O H Granulomatous thyroiditis (de Quervain s thyroiditis) J Clin Endocr 17 1202 1957

228 Woolner L B McConahey W M & Beahrs O H Struma lymphomatosa (Hashimoto s thyroiditis) and related thyroidal disorders J Clin Endocr 19 53 1959

229 Woolner L B McConahey W M Beahrs O H & Black B M Primary malignant lymphoma of the thyroid A review of forty six cases Amer J Surg 111 502 1966



To Astrid
Peter and Anna

CONTENTS

INTRODUCTION

It is well known that pancreatitis may cause overt diabetes. This applies especially to chronic pancreatitis, accompanied by calcification of the gland and steatorrhea. Even acute pancreatitis is sometimes followed by diabetes.

Decreased glucose tolerance without fasting hyperglycemia and glycosuria has often been demonstrated in chronic and sometimes also in acute pancreatitis. Little is known about glucose tolerance after the recovery from acute pancreatic disease.

It seemed probable that quantitative measurements of the capacity of the pancreas to produce and release insulin would give wider knowledge of the pancreatic endocrine function in pancreatitis. Methods for this purpose have recently become available through the studies of Cerasi and Luft (1963) and Cerasi (1967 a). The measurement of the insulin release during β-cell stimulation in patients with pancreatitis serves several purposes. It could make it possible to diagnose even minor malfunction of the β-cells and hence be used as additional evidence in the diagnosis of pancreatitis.

Few measurements of both endocrine and exocrine pancreatic function have hitherto been published. In the present study the degree of anatomical derangement in pancreatitis has been estimated by means of measurements of the pancreatic secretion after stimulation with high doses of secretin, in many instances combined with pancreozymin stimulation.

Aim of the Study

The following questions have been studied:

To what degree is insulin response to glucose stimulation altered in pancreatitis?

To what degree does pancreatitis impair exocrine pancreatic function?

Are endocrine and exocrine pancreatic functions correlated?

How to separate groups with different kinds of pancreatitis and normals from one another with the aid of pancreatic function studies?

Burton et al 1960 Plessier et al 1963, Bank et al 1963 Sun 1963 Rick 1964, Creutzfeld 1964) Dreiling and Janowitz (1962) have based on a vast experience, questioned the value of the addition of pancreozymin

In chronic pancreatitis, using secretin and/or pancreozymin stimulation decreased values were generally found for one or more of the variables — volume bicarbonate and enzymes After secretin stimulation Dreiling and Janowitz (Dreiling 1953 Dreiling and Janowitz 1956) obtained a quantitative deficiency which was most pronounced for bicarbonate less for enzymes and least for volume In the acute stage of acute pancreatitis secretin and pancreozymin tests have often shown decreased secretion of volume bicarbonate and enzymes For technical reasons it has however little diagnostic value A dissociation of enzyme secretion with selective decrease of amylase output has been demonstrated by Lagerlof (1942) Most authors are of the opinion that exocrine function becomes normal again a few weeks after the acute illness (Dreiling and Janowitz 1962)

Purified secretin (Jorpes and Mutt 1961) can be given in high doses without side effects This has made it possible to induce maximal or submaximal stimulation of the exocrine pancreas

Baron et al (1963) showed that maximal bicarbonate secretion in dogs was reached after a single injection of secretin in a dosage of 7 5 – 12 5 IU per kg body weight Supermaximum dosage of secretin gave a lower secretion of bicarbonate than a maximum dosage Lagerlof et al (1967) stimulated the human pancreas with 8 IU of secretin per kg body weight These authors are of the opinion that this dosage is about or somewhat below the dosage of maximum stimulation The same dosage has been used all through the present study

The maximal secretory capacity of the canine pancreas in response to pancreozymin and secretin was studied by Hansky et al (1964) Maximum total amylase secretion was obtained with 10 – 25 IU of pancreozymin per kg body weight as a single injection In each animal the maximum stimulation with secretin and pancreozymin gave reproducible values of volume as well as of bicarbonate and enzyme secretion

MATERIAL

The patients studied were gathered from 275 subjects treated for pancreatitis at the Surgical and Medical departments of Danderyd's Hospital during 1954 - 1964 Furthermore a small number of patients from Karolinska Hospital was included The patients were divided into two main groups one consisting of patients with chronic pancreatitis the other of those who had had one single attack of acute pancreatitis. Certain data on each patient are given in the Appendix

All studies of the patients within the two groups were carried out when there were no signs of acute pancreatitis

Chronic Pancreatitis

This group consisted of 1 patients who had been treated at Danderyd's Hospital 1954 - 1963 and who had had three or more typical acute attacks of pancreatitis of which at least two fulfilled the criteria of acute pancreatitis mentioned below 2 patients with pancreatic calculi and 3 four patients from Karolinska Hospital who fulfilled the diagnostic criteria given above Of 27 men and three women found in the records 24 men and the three women were examined The glucose infusion test (see Methods) was not performed in subjects on insulin treatment (no 1 2 4 6 7 11) because of eventual occurrence of insulin antibodies

Acute Pancreatitis

This group included patients who had been treated at Danderyd's Hospital 1954 - 1963 for acute pancreatitis

The diagnosis of acute pancreatitis was only accepted if there had been typical symptoms and signs of the disease and increased excretion of amylase in the urine (at least 512 Wohlgemuth-units) In many instances there were roentgenological signs of pancreatitis as well Out of these patients those born on one of the first five days of a month were selected Patients who had moved to another district were excluded Furthermore

patients born before 1890 were excluded as were a few patients with chronic diseases. Of the remaining seven men and ten women five men and eight women could be examined. The average age of these patients was 58 years. In order to obtain younger patients as well, those born in 1914 or later and treated in 1964 for acute pancreatitis at the Surgical Department of Danderyd's Hospital were called. Of four men and seven women four men and five women went through the examinations. Thus the whole group of acute pancreatitis finally included 10 men and 13 women. Two men and four women were 20–39 years old, four men and three women 40–59 years old, while three men and six women were over 60 years of age. Five subjects (no. 29, 30, 34, 35, 44) had a body weight exceeding 120 per cent of the average body weight (see Methods). The results of the glucose infusion test of these patients were excluded in the statistical analyses (Ikaram et al. 1963).

Control Group

The subjects of this group had no history of gastrointestinal disease or diabetes mellitus. They were chosen from voluntary blood donors and the hospital staff. The older age groups came from a home for aged people. The group consisted of 21 women and 29 men. Endocrine pancreatic function was studied in 28, and exocrine function in all of them.

Grouping and Designation

The different groups have been divided and designated as follows.

Control group (C)

- C_1 whole group
- C_2 age below 40 years
- C_3 age 40–59 years
- C_4 age 60 years or above
- C_5 females
- C_6 males

Chronic pancreatitis (Cp)

- Cp_1 whole group
- Cp_2 alcoholism present (no. 2–4, 6–10, 12, 17–22, 24–25)
- Cp_3 no alcoholism (no. 1, 5, 11, 13–16, 23, 26–27)
- Cp_4 overt diabetes (no. 1–14)
- Cp_5 no overt diabetes (no. 15–27)

Acute pancreatitis (Ap)

- Ap_1 whole group

Number, age, sex and body weight in kg and in per cent of average body weight in each of the groups are given in Table 1.

The term **alcoholism** has been used when the patient admitted at the author's interview a weekly consumption of at least one litre of spirits at the time of diagnosis of pancreatitis.

The term **diabetes** has been applied here only when the fasting blood-glucose concentration was repeatedly elevated, and glycosuria was present. Decreased glucose tolerance only was not termed diabetes.

TABLE 1 Data on groups and subgroups of controls and of chronic [illegible]
Data within brackets indicate values excluding subjects with a body weight [illegible]
average body weight calculated for sex age and body height

	Control group			
	whole group C_1	< 40 years C_2	40–59 years C_3	≥ 6[illegible] years C_4
number total	50	26	14	10
females	21	11	5	5
males	29	15	9	5
age years mean	39 8	23 8	46 1	72 [illegible]
range	19–77	19–36	40–59	69–7[illegible]
body wt kg mean	66 5	66 3	72 4	65 0
range	50–94	50–90	57–94	50–7[illegible]
average body wt per cent mean	95 8	96 6	93 3	90 7
range	80–114	84–112	86–114	80–102

	Groups with pancreati[illegible] chronic			
	whole group Cp_1	with alcoholism Cp_2	without alcoholism Cp_3	with dia[illegible] Cp_4
number total	27	17	10	14
females	3	0	3	1
males	24	17	7	13
age years mean	50 9	46 9	57 7	54 4
range	30–77	30–61	42–77	37–[illegible]
body wt kg mean	[illegible] 2	[illegible] 2	66 9	64 1
range	49–83	50–83	49–82	49–[illegible]
average body wt per cent mean	87 6	84 8	92 2	83 1
range	63 117	63–106	73–117	6[illegible]

METHODS

Material

Instruments

A continuous infusion aggregate (Mek Lab Konstruktioner, Västra Frolunda Sweden) was used for glucose infusions as well as the secretin-pancreozymin tests In the secretin-pancreozymin test a 50 ml syringe was used which gave 4 65 ± 0 02 ml per 15 min, and for the glucose infusion test two 300 ml syringes which together gave 87 0 ± 0 3 ml per 10 min

An Auto Gamma Spectrometer Model 314 (Packard Instrument Company, Ill , USA) was used in the radioimmunoassay of insulin and a general purpose analogue computer (E A I 131 R) in calculating the parameters of the glucose infusion test (*see below*)

The duodenal tube consisted of a double lumen tube (a duodenal part and a gastric part) with an exterior diameter of 8 mm to which was attached a tephlon tube for the leader (exterior and interior diameter 1 45 and 1 05 mm respectively) A short tube with many small perforations could also be attached for repeated local anaesthesia of the pharynx The duodenal part of the double lumen tube was 40 cm longer than the gastric part, and had in its lower 25 cm 17 big holes on one side and smaller holes on the other The tephlon tube emerged at the tip of the duodenal tube

The "leader" (Fig 1) consisted of a 4-5 m long spun nylon cord joined to a 60 cm soft plastic tube with a 10 cm long flaccid rubber bag containing shot and water at the end

Chemical compounds

Radioactive insulin was obtained by labelling crystalline insulin (30 IU per mg *from Nordisk Insulin AB* Copenhagen Denmark) with radioactive sodium iodide, $Na^{131}I$ (IBS, 3 The Radio Chemical Centre Amersham England)

Insulin antibodies were obtained by immunizing guinea-pigs with the crystal-

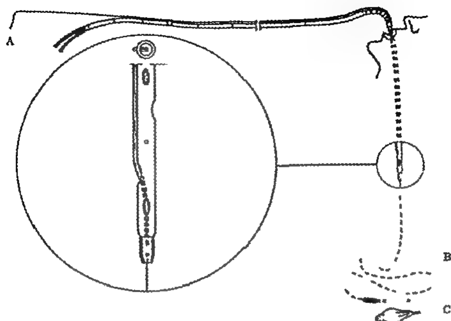


Fig 1 Illustration of the procedure of duodenal intubation A – C indicates the leader and B the stomach For further description see the text

line insulin mentioned The preparations of radioactive insulin as well as of insulin antibodies were prepared according to Hales and Randle (1963)

Sekretin® and Cecekin® (pancreozymin) were obtained from AB Vitrum Stockholm Sweden Both substances were kept at -20°C Sekretin® and Cecekin® are called in the following secretin and cecekin

Bromthalein® (Merck Darmstadt Germany) was used for the bromsulphthalein retention test and viscous Xylocain® (Astra Sodertalje Sweden) for local anaesthesia

The Intravenous Glucose Tolerance Test

The intravenous glucose tolerance test was performed according to Ikkos and Luft (1957) One hundred ml of a 25 per cent solution of glucose in water was given intravenously during three min Capillary blood was collected before and at every fifth min from 10 to 60 min and at 70 min after the start of the injection The disappearance rate of glucose (k-value) was calculated according to the following equation

$$k = \frac{0\ 693}{t_{\frac{1}{2}}} \quad 100$$

Time t was obtained by the graphical method from the curve where absolute glucose concentrations were plotted semilogarithmically against time

The Glucose Infusion Test (GIT)

Procedure

Plasma insulin response to glucose infusion was determined according to Cerasi and Luft (1963)

After an overnight fast glucose was given intravenously, firstly as a priming dose of 500 mg per kg body weight in a 25 per cent solution of glucose in water followed by a constant infusion of 20 mg glucose per kg body weight per min during the remainder of the hour Heparinized venous blood samples were collected through an indwelling needle before the glucose was given then at two 10-min intervals and thereafter every 20th min for the rest of three hours

Immunological and chemical analyses

Plasma-insulin was determined with a double-antibody radioimmunoassay according to Hales and Randle (1963) Glucose in the blood and urine was determined enzymatically with a commercial glucose-oxidase reagent (Kabi Reagent Stockholm Sweden)

The standard error of the mean (S E M) for the plasma insulin determinations was, within the same assay 1 3 and 2 0 per cent of the mean for low and high values, respectively The day-to-day variation was of the same magnitude with a S E M of 2 2 per cent (Cerasi 1967 b)

The relative experimental errors for the determinations of blood-glucose were 2 per cent and for those of glucose in the urine 3 per cent

Analogue computer analysis

Blood-glucose and plasma-insulin are intimately interrelated For evaluating plasma-insulin levels it is necessary to consider the synchronous levels of the blood-glucose An analogue computer is a suitable instrument for calculating such interrelationship In the present study an analogue computer model according to Cerasi (1967 a) was used

In order to obtain a usable and not too complicated model many physiological processes involved in the insulin - glucose interrelationship had to be simplified or omitted in the computer model For technical reasons, the computer worked only with changes in blood-glucose and plasma-insulin levels. When registering the results however the fasting values were added to the relative ones mentioned above

The computer model consisted of five systems (Fig 2)

System 1 registered the glucose con-

centration in the glucose space It integrated positive and negative inputs into the glucose pool and transformed the total amount of glucose in the glucose space to glucose concentration The positive input represented the priming injection and the infusion of glucose The negative inputs simulated the amounts of glucose transported from the glucose space partly through the kidneys into the urine and partly by the uptake of glucose into the peripheral tissues

System 2 simulated glucose loss through the kidneys For practical reasons the renal threshold for glucose had been set to 200 mg/100 ml The amount of glucose in the urine was equal to blood-glucose level above 200 mg/ 100 ml multiplied with a constant (k_u)

For technical reasons insulin release from the pancreas was simulated by two independent systems 3 and 4

System 3 simulated insulin release in response to the initial rise in blood-glucose The amount of insulin released was a function of the derivative of blood-glucose and a constant (k_{i1})

System 4 simulated insulin released after the initial phase Its function had a delay period of 15 min The system was stimulated by an increase in blood-glucose in relation to the coefficient k_{i2} The rate of insulin secretion was limited in order to fit the observed plasma-insulin curve (parameter b)

The amount of insulin released from systems 3 and 4 was corrected for its half-life

System 5 simulated glucose uptake

The reader is referred to Cerasi s report (1967 a) for the detailed computer circuit used as well as for the mathematical background of the model

The following parameters were obtained through analogue computation of the GIT

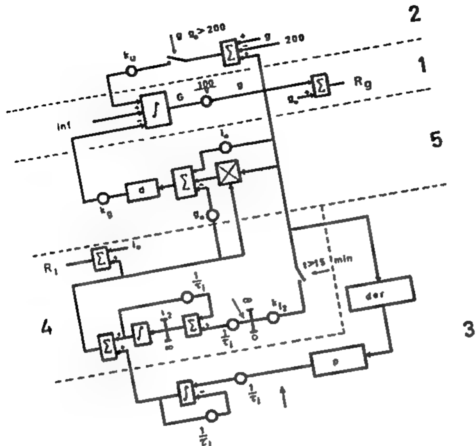
k_{i1} is the variable of system 3 and correlates the initial insulin release to the sudden increase in glucose concentration in the beginning of the GIT

k_{i2} correlates the later insulin secretion to the prevailing blood-glucose values Since plasma-insulin often continues to increase in spite of falling blood-glucose values k_{i2} tends to give high values in instances with a delayed insulin peak

b is the parameter corresponding to the rate of insulin secretion from system 4

k_g correlates the glucose uptake in peripheral tissues with the amount of insulin secreted It thus represents the biological activity of the released insulin

k_u is a constant correlating glycosuria to the blood-glucose concentration above 200 mg per 100 ml The figures of this constant are not presented in this



-○- multiplication by a constant

-⊠- multiplication by two variables

-[Σ]- summation

-[∫]- integration and summation

$\frac{k}{l}$ limiter k upper limit l = lower limit

m>n contact closed when the condition is fulfilled

2 Analogue computer model The numbers 1 to 5 refer to functions described in the text The arrows indicate the contact points from system 6 This figure is opied from Cerasi s report (1967 a) with due permission

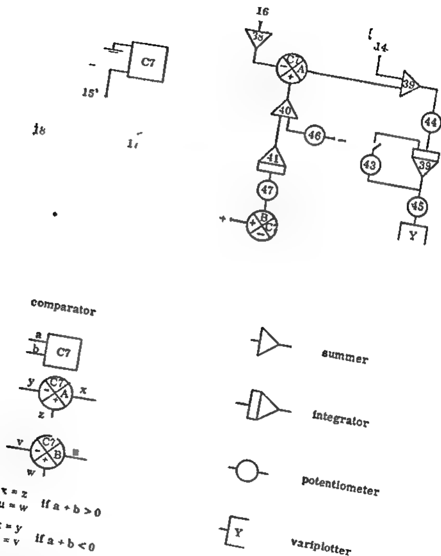


Fig 3 Detailed computer circuit for system 6 The numbers of the dotted symbols refer to corresponding numbers of the detailed analogue computer circuit reported by Cerasi (1967 a)

report as they do not characterize the different groups

V is the volume of distribution of glucose (glucose space) expressed in liter and in per cent of the body weight

Gu_{60} is glucose uptake during the first hour of the GIT, in g and in per cent of given amount (Gu_{60}%)

Gu_{180} is glucose uptake during the whole of the GIT, in g and in per cent of given amount (Gu_{180}%)

In addition to the parameters calculated according to Cerasi (1967 a) estimation of insulin secretion rate at any time of the GIT was obtained by the following modification of the computer model

Another unit system 6 (Fig 3) could be connected or disconnected to the model In system 6, the amounts of insulin released from systems 3 and 4 were added together before correction for the half-life of insulin A potentiometer (43) performing this correction could be connected to or switched off from the system

After simulation of the glucose and insulin curves system 6 was connected to the original computer model with the potentiometer 43 switched on The slope of plasma-insulin secretion was simulated with the aid of the potentiometers 46 and 47 Then potentiometer 43 was switched off and the insulin secretion rate in µU/ml per time unit was continuously and accumulatively recorded for the whole GIT period (see Fig 3) The insulin secretion rate per ml of plasma and per time unit was obtained from the curve (Ohlsén 1968) Insulin space was taken as equal to glucose space in the calculation of the amounts of insulin secreted

The following variables were determined

I_{30} I_{60} I_{90} I_{180} are the amounts of insulin secreted during 30, 60, 90 and 180 min respectively, and

I_{30}%, I_{90}% are the corresponding amounts of insulin in per cent of the total amount of insulin secreted

Discussion

In contrast to earlier insulin determinations during oral and intravenous glucose tolerance tests the GIT implies an effective and comparatively extended stimulation This gives possibilities of measuring the interaction between glucose concentration and insulin production under such conditions (Cerasi and Luft 1963) The mathematical development by Cerasi (1967 a) has made it possible to express in figures certain essential parameters of this interrelationship

With the aid of the GIT Cerasi and Luft (1967 b) have characterized prediabetes as a condition with an incapacity for an initial insulin response to glucose stimulation They assumed that genetic diabetes appears only in such subjects Overt diabetes would be precipitated

either by additional factors or by failure to compensate for the decreased sensitivity or capacity of the insulin releasing mechanism (Cerasi and Luft 1967 c)

It seemed likely that changes of the same mechanism might occur in pancreatitis or after such a disease and be of diagnostic significance

Glucose stimulation desirably should be of the same degree from person to person during different periods of the GIT For technical reasons this has not been obtainable In the initial phase of the test the glucose stimulation probably varies least from person to person Therefore the parameters characterizing the early phases of the test are more valid than those describing its later periods But in characterizing the insulin response during the GIT and in separating groups even those latter parameters may be of importance

The Secretin-Pancreozymin Test (SPT)

Duodenal intubation

Since 1962 the author has used a new method for duodenal intubation where the Seldinger method of arterial catheterization (Seldinger 1953) has been applied to intestinal intubation The duodenal tube is threaded on to a leader which previously has passed through the duodenum

The leader was introduced in two ways

1 the rubber bladder containing some lead-shot and fastened to a plastic tube was swallowed with a mouthful of water

2 the plastic tube was threaded into a plastic catheter of 5 mm diameter and was introduced as in an ordinary intubation When the rubber bladder reached the stomach 10 - 15 ml of water were injected through the inner plastic tube The external catheter was then removed The nylon cord already described was fastened to the plastic tube Surplus plastic tube was cut off and a short somewhat wider plastic tube was pushed over the knot to reduce the irritation to the pharynx Thereafter the leader could continue freely downwards with the aid of the peristaltic movements of the intestines

The leader was generally put down on the day before the experiment since most duodenal intubations started early in the morning At that time the leader as a rule had passed at least 1 2 m from the lower row of the teeth which was considered necessary before the intubation started

The duodenal tube used for the test was introduced into a fasting subject after premedication with 0 1 g pentobarbitalum and the pharynx anaesthetised locally with viscous Xylocain® During the introduction of the tube the patient

was lying on his right side on a fluoroscopy table with the feet about [illegible] cm lower than the head The nylon cord was threaded into the tephlon part of the duodenal tube This could easily be done by immersing the end of the cord and the distal tip of the tephlon tube in water and suction of water through the latter by means of a syringe The cord appeared together with the water When the duodenal tube reached the lips, the cord was stretched The tube was rubbed with viscous Xylocain® and pushed down the cord (see Fig 1) When the tip of the duodenal part had reached the ventricle the tube was held curved to facilitate the passage through the ventricle without coiling The tendency of coiling was further reduced by pulling the leader 5 - 10 cm

The first fluoroscopic examination was made when about 70 cm of the tube had passed the teeth If the form of the tube indicated that pylorus was passed, the tube was gently pushed forward until the end of the gastric lumen lied in the pyloric antrum If the tip had not passed pylorus the tube was pushed further It usually passed the pylorus after a few manipulations The position was checked by fluoroscopy as well as X-ray In a few uncertain cases air was injected through the duodenal and ventricular tubes in order to improve the visualization of the pylorus At the end of the test the cord was washed down the intestines with water

This procedure was used in more than 100 duodenal intubations with only one failure In this case the tube had been put down without being checked by fluoroscopy and had formed a knot in the ventricle The pylorus was passed within 5 - 65 min (mean 14 min) and the automatically measured fluoroscopy time was 10 - 170 sec (mean 44 sec) The tube in place the patient was transferred to a comfortable bed for the test

Technical procedures

Hypersensitivity towards secretin and cecekin was tested with an intracutaneous test

The duodenal juice was collected by siphonage under careful control The gastric juice was sucked up with water suction apparatus with a negative pressure of 15 mm Hg The duodenal and ventricular juices were collected in ice cooled containers Special suction of the saliva was applied when necessary

After 20 min fasting samples were collected during a 15 min period Secretin was then administered in a dosage of 1 IU per kg body weight It was given intravenously for [illegible] - [illegible] min through an indwelling needle which was kept open by a slow infusion of saline Fifteen min after the injection of secretin another 7 5 IU of secretin per kg body weight were given intravenously for

3 - 5 min The collection of duodenal and ventricular juices continued for another four 15-min periods Finally 1 IU of cecekin per kg body weight was given intravenously for 3 - 5 min and another two 15-min samples were collected

The duodenal and gastric juices from each period were measured Five ml of duodenal juice from each period were mixed with [illegible] ml of ice cooled glycerine in a shaking apparatus for determination of bicarbonate amylase trypsin and bilirubin

If the amount of gastric juice for one period was larger than 10 ml, a reflux of duodenal juice was suspected to have occurred In these cases the same analyses were applied to the gastric as to the duodenal juice Attempts were made to estimate the quantity of duodenal juice which was brought up via the gastric tube by comparing the concentration of bilirubin in the duodenal and gastric juices

All samples were kept at -20°C until the determinations were performed either on the day of the test or on one of the following days

Chemical analyses

Amylase in the duodenal juice was determined according to Lagerlof (1942) trypsin according to Erlanger et al (1961) bicarbonate by indirect titration with 1/10 of normal hydrochloric acid and sodium hydroxide using phenolphtalein as an indicator and bilirubin according to Jendrassik and Grof (1938)

The relative experimental errors for the determinations of bicarbonate ; amylase and trypsin were 5 3 and 7 per cent respectively The fall in bicarbonate levels and amylase and trypsin activities when the samples were kept at -20° C for 12-52 (mean 33) weeks were determined The mean values and ranges were for bicarbonate [illegible] 37 (-0 42 to +1 83) mE/l per week amylase 0 003 (-0 06 to +0 04) AU/ml per week and trypsin 2 2 (-5 7 to +11 3) µg/ml per week

Discussion

The author has introduced a new procedure for duodenal intubation This method allows duodenal intubation to be performed within about 15 min and also increases considerably the number of successful intubations In fact in the author s hands the intubation was successful in every single experiment save one It should be pointed out that many authors have considered the intubation a major stumbling-block in the diagnosis of pancreatic disease connected with secretin or pancreozymin tolerance tests Recently McGillivray et al (1966) reported failures in four intubations out of 13 even

when the intubations were extended for four hours The majority of successful intubations required about two hours for the introduction of the tube into the duodenum

The author has calculated the quantities of products secreted In order to make all these calculations as accurate as possible, it is required that the whole of the quantity of juice secreted is collected or that the collected portion of that quantity can be calculated For this purpose attempts were made by Bartelheimer (1955) to obstruct the duodenum with balloons on either sides of the apertures in the duodenal part of the tube It has not been shown that such a procedure guarantees the collection of the whole quantity secreted Other authors have used reference substances in order to calculate how much of the secreted quantity is recovered In the present study the author constantly used radioactive B_{12} as a reference substance according to the method described by Schutz and Reizenstein (1963) Later calculations showed however that the variation coefficients for different variables did not decrease when this procedure was used This might be due to the fact that the B_{12} solution was infused into the intestine too close to the pylorus For this reason the results of these calculations have not been reported

It is well known from earlier reports with other secretin preparations that side effects may appear already with small quantities of the drug In the present study very few side effects have been noted with secretin dosages of 8 IU per kg body weight — in single instances rapidly passing flushes or palpitations With the majority of patients pancreozymin caused slight to moderate malaise palpitations, or cramps in the epigastrium In one patient the blood pressure fell considerably but could easily be raised These side effects, forced the author to refrain from maximal pancreozymin stimulation and conventional dosages of one unit per kg body weight were used

No patients showed intracutaneous hypersensitivity towards secretin or pancreozymin

It is well known that the activity of secretin varies within and between the different preparations Such variations ought to be less pronounced when high dosages are used

This study shows that the procedure of duodenal intubation introduced by the author in many respects is superior to previous methods Furthermore the amount of secretin administered can be considered as appropriate for the purpose Cecekin because of side effects, could not be given in as high dosages as was desired

Other methods

Glutamic oxalacetic transaminase (GOT) glutamic pyruvic transaminase (GPT) and alkaline phosphatases were determined with commercial reagents (Kabi Reagent Stockholm Sweden) The bromsulphthalein retention test was determined by calculation of the dose according to Brohult (1967) and determination of the plasma concentration of dye according to Gaebler (1945)

Body surface area and lean body mass were calculated from body weight and height according to Du Bois and Du Bois (1916) and Hume (1966) respectively Average body weight in comparable populations used in pages 12 and 13 was determined from age sex and body height (Documenta Geigy 1960)

Statistical determinations were mainly performed according to Snedecor (1964) The following probability (P) levels were used $P < 0.001$ (***) highly significant $0.001 < P < 0.01$ (**) significant and $0.01 < P < 0.5$ (*) probably significant The means were generally presented ± the standard error of the mean (S E M) In all comparisons between two groups the difference indicated a subtraction of the latter value from the former

Computations of correlations between different variables were performed according to Zackrisson and Reizenstein (1967)

The experimental error (s) was calculated as follows

$$s = \sqrt{\frac{\Sigma d^2}{2n}}$$ where

d is the differences between the values of (n) paired estimations

The relative experimental error (C) was determined in the following way

$$C = {}^{e}\log 10 \quad s_L = 2\ 3026 \quad s_L$$ where

$$s_L = \sqrt{\frac{\Sigma L^2}{2n}}$$ and

L = differences between the logarithmic values of paired estimations

Discriminant analysis

Separations were performed with the use of one or several variables between following groups

control group (C) and group with chronic pancreatitis (Cp)

C and group with acute pancreatitis (Ap)

Ap and Cp

Discriminations with several variables were carried out according to Anderson (1958) This method presupposes that all values in the covariance matrix are equal between the groups This presumption is fulfilled if the variances for each variable and the coefficients of correlation are equal between the three groups

The variances were compared with Bartlett's test (Hald 1960) according to the following equation

$$(1)\ \chi^2 = \frac{2.3025}{c}\left[(N - m)\log s^2 - \sum(n_i - 1)\log s_i^2\right]$$

where $s_1, s_2, s_3, \ldots s_m$ are the variances in the groups i (1, 2, ... m) and $n_1, n_2, n_3, \ldots n_m$ are the numbers of observation in the groups

$$N = \sum n_i$$

$$c = 1 + \frac{1}{3(m-1)}\left(\sum \frac{1}{(n_i - 1)} - \frac{1}{N - m}\right)$$

$$s^2 = \frac{\sum(n_i - 1)s_i^2}{N - m}$$

The significance was received from a table of the χ^2-distribution with m - 1 degrees of freedom. All variances were tested, and only the variances of the following variables differed between the groups: Gu_{180}% ($P < 0.001$), k-value ($P < 0.05$) and log b ($P < 0.05$).

The coefficients of correlation were compared according to the following equation (Hald 1960)

$$(2)\quad u = \frac{z_1 - z_2}{\sqrt{\frac{1}{n_1 - 3} + \frac{1}{n_2 - 3}}}$$

where

$$z_i = 1.1513 \log \frac{1 + r_i}{1 - r_i}$$

r_i = coefficient of correlation for two variables in group i

n_i = number of observations in group i

u is normally distributed.

The comparisons were performed between 15 randomly chosen coefficients of correlation between the variables chosen for discriminant analysis.

Out of 45 comparisons only the following three differed significantly:

1. between C and Cp regarding the correlation between Gu_{180}% and I_{90}% ($P < 0.05$)
2. between C and Ap ($P < 0.01$), and
3. between Cp and Ap ($P < 0.05$), both regarding the correlation between log b and the amount of amylase recovered during 60 min after giving secretin and corrected for body weight.

Thus, it was reasonable to presume that the covariance matrix was about the same for the three groups and consequently that the prerequisite for discriminant analysis was fulfilled. The only reservation was calculations including the variable Gu_{180}%, where the differences between the groups were more pronounced than for other variables. The reason for that was probably the small variance in the control group.

The discriminant function (d) is a value calculated for each individual with regard to the values for all variables applied and with special reference to the abilities of these variables to separate two groups. The discriminant function was computed in the following way:

(3) $d = \underline{x}'\Sigma^{-1}(\mu_1 - \mu_2)$ where

$\underline{x}'$ = the observed values of the variables written as a vector

Σ^{-1} = the inverted covariance matrix

μ_1 = the vector of all means of all variables in group 1

Developing (3) one gets

$d = x_1 f_1 + x_2 f_2 + \quad x_n f_n$ where x_n is the observed value of the n-th variable and f_n is the coefficient of the variable x_n in the discriminant function

For three variables x_1, x_2 and x_3 and two groups 1 and 2 the above equation could be expressed as follows

$d = \underline{x}'\Sigma^{-1}(\mu_1 - \mu_2)$ where

$$\underline{x}' = \begin{bmatrix} x_1 & x_2 & x_3 \end{bmatrix}$$

$$\mu_1 = \begin{bmatrix} \mu_{1_1} \\ \mu_{1_2} \\ \mu_{1_3} \end{bmatrix} \qquad \mu_2 = \begin{bmatrix} \mu_{2_1} \\ \mu_{2_2} \\ \mu_{2_3} \end{bmatrix}$$

$$\mu_1 - \mu_2 = \begin{bmatrix} \mu_{1_1} - \mu_{2_1} \\ \mu_{1_2} - \mu_{2_2} \\ \mu_{1_3} - \mu_{2_3} \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}$$

The covariance matrix with its covariances (c_{ij}) and variances (σ_i^2) can be written as follows

$$\Sigma = \begin{bmatrix} \sigma_1^2 & c_{12} & c_{13} \\ c_{12} & \sigma_2^2 & c_{23} \\ c_{13} & c_{23} & \sigma_3^2 \end{bmatrix}$$

The inverted covariance matrix can be written in the following way

$$\Sigma^{-1} = \frac{1}{A}\begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{12} & a_{22} & a_{23} \\ a_{13} & a_{23} & a_{33} \end{bmatrix}$$ where

$$a_{11} = \sigma_2^2\sigma_3^2 - c_{23}^2$$

$$a_{22} = \sigma_1^2\sigma_3^2 - c_{13}^2$$

$$a_{33} = \sigma_1^2\sigma_2^2 - c_{12}^2$$

$$a_{12} = c_{23}c_{13} - \sigma_3^2 c_{12}$$

$$a_{13} = c_{12}c_{23} - \sigma_2^2 c_{13}$$

$$a_{23} = c_{12}c_{13} - \sigma_1^2 c_{23}$$

and

$$A = \sigma_1^2 a_{11} + c_{12}a_{12} + c_{13}a_{13} =$$

$$= \sigma_1^2\sigma_2^2\sigma_3^2 - \sigma_1^2 c_{23}^2 - \sigma_2^2 c_{13}^2 -$$

$$- \sigma_3^2 c_{12}^2 + 2c_{12}c_{13}c_{23}$$

$d = \underline{x}'\Sigma^{-1}(\mu_1 - \mu_2)$ can then be written as follows

$$d = x_1\frac{a_{11}v_1 + a_{12}v_2 + a_{13}v_3}{A} +$$

$$+ x_2\frac{a_{12}v_1\, a_{22}v_2 + a_{23}v_3}{A} +$$

$$+ x_3\frac{a_{13}v_1 + a_{23}v_2 + a_{33}v_3}{A}$$

The borderline value (b) (Fig 4) is a value of d above which a subject will be classified to one group and below which

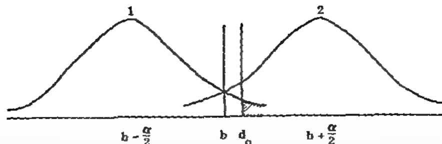


Fig 4 Illustration of the distributions of the discriminant function The shaded area shows the probability of obtaining a value of the discriminant function d_o or higher when the subject actually belongs to group 1

he will be classified to another This value is calculated as follows

$$(4)\quad b = \log \frac{q_2\, c(1|2)}{q_1\, c(2|1)} + \frac{1}{2}(\mu_1 + \mu_2)' \Sigma^{-1} (\mu_1 - \mu_2)$$

where

q_1 is the probability of finding a subject belonging to group 1 in the population in which it is reasonable to perform these investigations,

$c(1|2)$ and $c(2|1)$ = the costs (clinical disadvantages) of misclassifying a subject belonging to the groups 2 and 1 as belonging to the groups 1 and 2 The values of q and c are unknown It may be reasonable to assume that

$q_2\, c(1|2) = q_1\, c(2|1)$ then

$\log \frac{q_2\, c(1|2)}{q_1\, c(2|1)}$ in equation (4) is 0

At separation between two groups with the aid of one variable this variable was assumed to have the same variance in both groups as that was a prerequisite for the above mentioned method of discriminant analysis Thus the separation value between the groups became the mean of the mean values of each group

The different selections of discrimination were evaluated in the following ways (see Tables 22 and 23)

The probabilities (P) of misclassification a subject with a value of the variable — or in case of several variables a value of the discriminant function — equal to the above mentioned borderline value (b), symbolized the value of each selection These probabilities were calculated according to following equations

a) using one variable

$P(1|2) = P(2|1) =$

$$= \Phi \left[\frac{\log \frac{q_2\, c(1|2)}{q_1\, c(2|1)} - \frac{1}{2}(\mu_1 - \mu_2)^2 \frac{1}{\sigma^2}}{\frac{\mu_1 - \mu_2}{\sigma}} \right]$$

and assuming that $\log \frac{q_2 c(1|2)}{q_1 c(2|1)}$ 0

(5) $P(1|2) = P(2|1)$ $\Phi\left(\frac{1}{2}\frac{\mu_1 - \mu_2}{\sigma}\right)$

where

$P(1|2)$ and $P(2|1)$ = the probabilities that a subject belonging to the groups 2 and 1 respectively is misclassified to groups 1 and 2

μ_1 and μ_2 = mean for this variable in groups 1 and 2

σ average standard deviation in groups 1 and 2 reckoned according to the equation

(6) $$\sigma \quad \sqrt{\frac{(n_1 - 1)\sigma_1^2 + (n_2 - 1)\sigma_2^2}{(n_1 - 1) + (n_2 \quad 1)}}$$

Φ the distribution function of the normal distribution

b) using several variables

$$P(1|2) \quad P(2|1) \quad \Phi\left[\frac{\log \frac{q_2 c(1|2)}{q_1 c(2|1)} - \frac{1}{2}\alpha}{\sqrt{\alpha}}\right]$$

and for $\log \frac{q_2 c(1|2)}{q_1 c(2|1)} = 0$

(7) $P(1|2) \quad P(2|1) = \Phi\left(-\frac{1}{2}\sqrt{\alpha}\right)$

where

$\alpha \quad (\mu_1 \quad \mu_2)' \Sigma^{-1} (\mu_1 \quad \mu_2)$

A simple way of estimating the relative value of the different selections of discriminations was by comparing the number of subjects with pancreatitis misclassified as normals and vice versa This was done by introducing the classification in tabular form (see Table 23)

There is a possibility of estimating the accuracy of a classification Assume that in Fig 4 group 1 represents normals and group 2 subjects with pancreatitis The probability (P_1) of obtaining a value (d_o) of the discriminant function higher than b can be calculated as follows

(8) $P_1 \quad 1 - \Phi\left(\frac{d_o + \frac{\alpha}{2} - b}{\sqrt{\alpha}}\right)$

α is explained in (7)

The probability (P_2) of obtaining a value (d_o) of the discriminant function lower than b can be calculated in the following way

(9) $P_2 \quad \Phi\left(\frac{d_o - \frac{\alpha}{2} - b}{\sqrt{\alpha}}\right)$

The accuracy of the classification is higher the lower the values are for P_1 and P_2

The same equations may be applied to calculate corresponding probabilities using one variable if α is equal to

$\frac{(\mu_1 - \mu_2)^2}{\sigma^2}$ where

μ_1 μ_2 and σ are explained in (5)

RESULTS

The primary data obtained are presented in the Appendix

The Intravenous Glucose Tolerance Test

The occurrence of overt diabetes and decreased glucose tolerance (k-value <0 95) (Lundbaek 1962) in the control group (C) and the groups of chronic (Cp) and acute (Ap) pancreatitis is presented in Table 2

In the control group the mean k-value was 1 54 ± 0 11 There were no significant differences between the k-values in the three age groups (<40, 40 - [illegible] and ≥ 60 years) The *correlations* of the k-values to age, body measurements and lean body mass are presented in Table 3 The k-value decreased with increasing age, body measurements and lean body mass but the correlations were not significant

The mean k-value in the group with chronic pancreatitis was 0 86±0 09, and in the patients after acute pancreatitis 1 34±0 08 In the former group

TABLE [illegible] Occurrence of overt diabetes, decreased and normal glucose tolerance in the control group and the groups with chronic and acute pancreatitis

	C	Cp	Ap
overt diabetes		14	
decreased glucose tolerance *only*	2	6	4
normal glucose tolerance	26	7	19
total number	[illegible]	27	[illegible]

there was no significant difference in k-values between the subgroups with and without alcoholism

The mean k-values of the control group and the group of acute pancreatitis did not differ significantly, while both values were significantly higher than that of the group with chronic pancreatitis (P < 0 001)

The correlations between the k-values and other variables of endocrine pancreatic function in the control group and the groups with chronic and acute pancreatitis are presented on page 37

The Glucose Infusion Test (GIT)

Distribution of data

The values for the following variables of the GIT – k_{i1}, k_{i2}, b and k_g – in the control group and in the two groups with pancreatitis, when plotted on normal distribution paper did not show a normal distribution The distribution of the logarithms was less skew than that of the absolute values Therefore logarithmic values were used throughout this presentation In the following log k_{i1} stands for log $k_{i1} \times 10^3$, log k_{i2} for log $k_{i2} \times 10^2$, and log b for log b $\times 10^2$

When the values of the following variables of the GIT – I_{30}, I_{60} and I_{180} – in the three groups were plotted on normal distribution paper, the lines were straighter than corresponding lines for the logarithmic values Therefore, the non-transformed values of these variables were used

Control group

Influence of age body measurements and lean body mass on the GIT

The correlations obtained are presented in Table 3 and the regression lines of log k_{i1} and Gu_{60}% on age in Fig 5

There was a highly significant inverse correlation between glucose uptake during 60 min in per cent of given amount (Gu_{60}%) and age ($P < 0.001$) The ratio between glucose uptake during

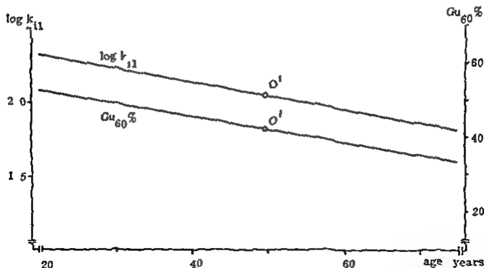


Fig 5 The regression lines of log k_{i1} and Gu_{60}% on age O' are log k_{i1} 2 043 age 49 6 years and Gu_{60}% 42 0 age 49 6 years The sample regression equations are $y = -0.0094297x$ for log k_{i1} and $y = -0.35336x$ for Gu_{60}%

TABLE II Correlations between variables of endocrine pancreatic function versus age, measurements and lean body mass $Gu_{180-60/60}$ denotes the ratio between glucose upt during the last two and the first hour of the GIT

	n	Age	weight	Body height	surface area	L bo m
$\log k_{i1}$	28	-0 33 *	-0 01	-0 05	-0 04	-0
$\log k_{i2}$	26	-0 35	0 45 *	0 38 *	0 43 *	0 *
log b	28	-0 23	0 24	0 07	0 17	0
Gu_{60}%	28	-0 62 ***	0 07	0 29	0 18	0
Gu_{180}%	27	-0 50 *	0 30	0 37	0 35	0
$Gu_{180-80/60}$	22	0 54 **	0 39			
I_{30}	22	-0 29	-0 14			
I_{60}	22	-0 26	-0 02			
I_{180}	22	-0 27	0 38			
I_{30}%	22	-0 20	-0 43 *			
I_{60}%	22	-0 11	-0 45 *			
I_{90}%	22	-0 08	-0 36			
Glucose space in l	28		0 64 ***			
Intravenous glucose tolerance test k-value	28	-0 28	-0 16	-0 14	0 16	-0 19

the times 60 - 180 min and 0 - 60 min ($Gu_{180-60/60}$) was also found to be correlated to age ($P < 0\ 01$) The correlation between glucose space in liter and body weight was highly significant ($P < 0\ 001$) The correlation was less significant between Gu_{180}% and age ($P < 0\ 05$) $Log\ k_{i2}$ showed some correlation to body weight and height, body surface area and lean body mass ($P < 0\ 05$) No significant correlations were found between $\log k_{i1}$ and body measurements

Thus, age body measurements and lean body mass had an influence on some parameters of the GIT The effect of age is noticeable

Measurements of parameters of the GIT

The mean values are presented in Tables 4 and 5 and the distributions of the values of some parameters in Fig 6

In the control group two subjects (7%) had values of log k_{11} < 1 0 and six (21 %) had values < 1 8 an arbitrarily chosen border value between low and high responses (Cerasi and Luft 1967 a)

Comparisons between the age groups and between females and males are shown in Table 6 (the line log k_g in the table should be deleted) The differences between the three age groups C_2 (< 40 years) C_3 (40 - 59 years) and C_4 (≥ 60 years) were as follows C_2 had a significantly higher glucose uptake during 60 min in per cent of given amount than group C_4 ($P < 0\ 01$) Log k_{11} and Gu_{180}% were probably significantly higher in C_2 than C_4 ($P < 0\ 05$) Log b was probably significantly higher in C_2 than C_3 The glucose uptakes during 60 and 180 min in per cent of given amount were probably higher in C_3 than C_4 ($P < 0\ 05$)

The female group had probably significantly higher log k_{11} than the male group ($P < 0\ 05$)

In conclusion, there were significantly lower values for glucose uptakes during 60 and 180 min in per cent of given amount in the older age groups and there seemed to be a tendency in these groups towards lower values for log k_{11} and log b

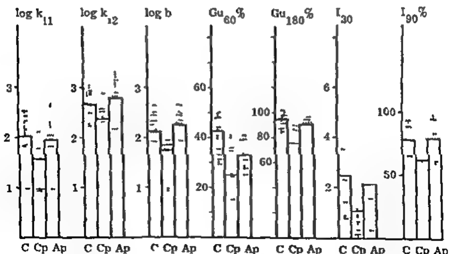


Fig 6 Distributions of parameters of the GIT in the control group (C) and the groups with chronic (Cp) and acute (Ap) pancreatitis

TABLE 4 Measurements of parameters of the GIT in the different groups of subjects

		Volume of glucose space		log k_{11}	log k_{12}
		l	% of body wt		
Control groups					
C_1 whole group	n = 29	18 4 ± 0 6	27 8 ± 0 8	[illegible] 043 ± [illegible] 090	[illegible] 690 ± [illegible] 052
C_2 < 40 years	n = 7	17 8 ± 1 1	27 8 ± 1 8	2 346 ± 0 100	2 818 ± 0 057
C_3 40 - 59 years	n = 10	19 0 ± 0 8	27 1 ± 1 0	2 013 ± 0 131	2 704 ± 0 076
C_4 ≥ [illegible] years	n = 11	18 4 ± 1 2	28 5 ± 1 4	1 877 ± 0 173	2 590 ± 0 102
C_5 females	n = 13	16 8 ± 0 6	28 4 ± 1 1	2 232 ± 0 124	2 628 ± 0 065
C_6 males	n = 15	19 8 ± 0 7	27 3 ± 1 1	1 879 ± 0 118	2 743 ± 0 079
Groups with chronic pancreatitis					
Cp_1 whole group	n = 20	18 6 ± 0 6	27 9 ± 0 9	1 543 ± 0 112	[illegible] 366 ± 0 117
Cp_2 with alcoholism	n = [illegible]	18 8 ± 0 8	28 4 ± 1 0	1 [illegible]1 ± 0 117	2 308 ± 0 158
Cp_3 without alcoholism	n = 7	18 3 ± 0 9	27 0 ± 1 6	1 881 ± 0 183	2 491 ± 0 144
Cp_4 with diabetes	n = 7	18 8 ± 1 1	28 2 ± 1 1	1 287 ± 0 174	2 161 ± 0 254
Cp_5 without diabetes	n = 13	18 6 ± 0 7	27 8 ± 1 3	1 682 ± 0 134	2 485 ± 0 107
Group of acute pancreatitis					
Ap_1 whole group	n = 18	18 3 ± 0 9	24 9 ± 0 8	1 977 ± [illegible] 107	2 779 ± 0 065

log b	log [illegible]$_g$	Glucose uptake during			
		60 min		180 min	
		[illegible]	% of given amount	g	% of given amount
2 159 ± 0 069	1 464 ± 0 039	46 9 ± 3 0	42 0 ± 2 2	105 6 ± 3 8	94 8 ± 0 9
[illegible] 356 ± 0 071	1 501 ± 0 051	55 8 ± 8 6	51 2 ± 4 7	103 4 ± 7 2	96 9 ± 1 4
2 066 ± 0 100	1 528 ± 0 062	51 5 ± 3 5	44 4 ± 3 4	114 9 ± 7 0	96 3 ± 1 6
2 119 ± 0 140	1 358 ± 0 067	36 9 ± 2 6	34 2 ± 1 4	99 4 ± 5 2	92 1 ± 1 1
2 214 ± 0 103	1 405 ± 0 045	40 4 ± 2 6	41 2 ± 2 2	92 0 ± 3 4	[illegible] 1 ± 1 3
2 111 ± 0 091	1 516 ± 0 060	52 4 ± 4 8	42 8 ± 3 6	118 3 ± 4 4	95 4 ± 1 2
1 719 ± 0 104	1 326 ± 0 050	27 0 ± 2 7	25 0 ± 2 5	83 0 ± 5 7	76 3 ± 4 8
1 634 ± 0 144	1 285 ± 0 065	22 6 ± 3 4	21 2 ± 3 1	76 5 ± 7 5	71 2 ± 6 3
1 876 ± 0 118	1 416 ± [illegible] 068	35 0 ± 2 4	32 1 ± 3 0	97 2 ± 3 9	87 3 ± 4 2
1 432 ± 0 198	1 250 ± [illegible] 064	20 8 ± 4 3	[illegible] 6 ± 3 7	72 7 ± 12 2	64 9 ± 9 7
1 873 ± 0 099	1 371 ± 0 069	30 3 ± 3 1	28 5 ± 3 0	89 0 ± 5 2	82 9 ± 4 2
2 261 ± 0 063	1 303 ± 0 040	33 2 ± 2 8	31 5 ± 1 5	109 1 ± 5 5	90 2 ± 1 4

TABLE 5 Measurements of parameters of the GIT in the control group and the groups with chronic and acute pancreatitis

	I_{30}	I_{60}	I_{180}	I_{30}%	I_{90}%	Gu_{180} - 60/60
Controls n 22	2 48 ± 0 36	5 84 ± 0 67	11 03 ± 1 08	21 8 ± 2 6	85 7 ± 3 3	1 39 ± 0 09
Group of chronic pancreatitis n 18	1 05 ± 0 30	2 95 ± 0 74	8 64 ± 1 50	11 2 ± 1 8	61 5 ± 5 2	2 46 ± 0 22
Group of acute pancreatitis n 18	2 14 ± 0 53	6 11 ± 1 08	14 04 ± 2 45	13 6 ± 1 2	80 7 ± 3 4	1 91 ± 0 11

TABLE 6 Comparisons between the age groups (C_2 C_3 C_4) and between females (C_5) and males (C_6) of some variables of the GIT

	C_2 - C_3 n		C_2 - C_3 difference/ level of significance	C_2 - C_4 n		C_2 - C_4 difference/ level of significance	C_3 - C_4 n		C_3 - C_4 difference/ level of significance	C_5 - C_6 n		C_5 - C_6 difference/ level of significance
	C_2	C_3		C_2	C_4		C_3	C_4		C_5	C_6	
log k_{11}	7	10	0 34	7	11	0 47 *	10	11	0 13	13	15	0 35 *
log k_{12}	7	10	0 12	7	11	0 22	10	11	0 10	13	15	-0 11
log b	7	10	0 29 *	7	11	0 24	10	11	-0 05	13	15	0 11
log k[illegible]	7	10	-0 02	7	11	0 28	10	11	0 30 *	13	15	-0 24
[illegible]	[illegible]	[illegible]	6 8	7	11	17 1 **	10	11	10 3 *	13	15	-1 6
[illegible]				[illegible]	11	4 8 *	9	11	4 2 *	13	14	-1 2

Correlations between parameters of endocrine pancreatic function

The correlations obtained are shown in Table 7

There were highly significant correlations between $\log k_{11}$ and log b and also between both of these on one side and variables of amounts of insulin secreted at various times on the other ($P < 0.001$) The k-value was found to be correlated to $\log k_{11}$ ($P < 0.01$)

The above results indicate that in normal subjects there are several intimate interrelations between the parameters of endocrine pancreatic function presented

Groups of chronic and acute pancreatitis

Measurements of parameters of the GIT

Comparisons between the two groups with pancreatitis are presented on page 42

The mean values of the parameters of the GIT are presented in Tables 4 and 5 and the distribution of the values of some parameters in Fig 6

In the group of chronic pancreatitis seven subjects (35%) had values of $\log k_{11} < 1.0$ Four of these had overt diabetes Fourteen (70 %) subjects had values < 1.8 and six of these had overt diabetes One patient with overt diabetes had $\log k_{11} > 1.8$

In the group of chronic pancreatitis the subgroup with alcoholism in comparison to the one without alcoholism, had lower absolute values for all variables The former group had significantly lower values for $\log k_{11}$ $Gu_{60}\%$ and $Gu_{180}\%$ ($P < 0.05$) than the latter one

In the group of acute pancreatitis two subjects (9 %) had values of $\log k_{11} < 1.0$ and eight (35 %) had values < 1.8

Correlations between parameters of endocrine pancreatic function

The significant correlations obtained are presented in Tables 8 and 9

In both groups of pancreatitis the correlations were of about the same frequency and level of significance as in the control group $Gu_{180}\%$ $\log k_{11}$ and log b seemed to be the parameters with the most significant correlations to other variables

Comparisons of some variables of the GIT between the control group and the groups of chronic and acute pancreatitis

The results of these studies are presented in Table 10 Typical curves of the results of the GIT in a subject from each of the three groups are shown in Fig 7

The fasting blood-glucose and plasma-insulin values were compared between the three groups The group with chronic pancreatitis had a probably significantly higher value of fasting blood-glucose than both the control group ($P < 0.02$) and the group of acute pancreatitis ($P < 0.05$) All other

TABLE 7 Correlations between parameters of endocrine pancreatic function in the control group

	n	log k_{11}	log k_{12}	log b	Gu_{60}%	Gu_{180}%	$Gu_{180-60/60}$	I_{30}	I_{60}	I_{180}	I_{30}%	Intravenous glucose tolerance test k-value
n		28	28	28	28	27	22	22	22	22	22	
log k_{11}	28		0.48**	0.79***		0.47*		0.80***	0.77***	0.55**	0.69***	0.54**
log k_{12}	28	0.48**		0.62***		0.47*				0.64**		
log b	28	0.79***	0.62***			0.38*		0.73***	0.92***	0.79***	0.46*	
Gu_{60}%	28					0.55**	-0.97***	0.44*			0.48*	0.46*
Gu_{180}%	27	0.47*	0.47*	0.38*	0.55**							
Gu_{180} - 60/60	22				-0.97***			-0.43*			-0.57**	-0.46*
I_{30}	22	0.80***		0.73***	0.44*		-0.43*		0.88***	0.58**	0.84***	
I_{60}	22	0.77***		0.[illegible]***				0.88***		0.77***	0.59**	
I_{180}	22	0.55**	0.64**	0.79***				0.58**	0.77***			
I_{30}%	22	0.69***		0.46*	0.48*		-0.57**	0.84***	0.59**			
					0.46*		-0.46*					

TABLE 9 C rrelatio s betwee [illegible] rameters of en [illegible] rine [illegible] ancre atic function in the group of acute [illegible] ncreatitis

	n	log k_{11}	log k_{12}	log b	Gu_{60}%	Gu_{180}%	Gu_{180} 60/60	I_{30}	I_{60}	I_{180}	I_{30}%	iv glucose tolerance test, k-value
n		18	18	[illegible]	18	[illegible]	18	18	[illegible]	[illegible]	18	
log k_{11}	18		0 80 ***	0 69 **		0 73 ***		0 79 ***	0 77 ***	0 76 ***	0 61 **	
log k_{12}	18	0 80 ***		0 69 **		0 61 [illegible]		0 62 **	0 68 **	0 62 **		
log b	18	0 69 **	0 69 **			0 57 *		0 62 **	0 79 ***	0 72 ***		0 48 *
Gu_{60}%	18					0 73 ***	-0 81 ***				0 74 ***	
Gu_{180}%	18	0 73 ***	0 64 **	0 57 *	0 79 ***		0 51 *	0 51 *	0 63 *		0 72 ***	
Gu_{180} 60/60	[illegible]				0 89 ***	-0 51 *					-0 48 *	-0 51 *
I_{30}	18	0 79 ***	0 62 **	0 62 **		0 51 *			0 91 ***	0 94 ***	0 60 **	
I_{60}	18	0 77 ***	0 68 **	0 79 ***		0 53 *		0 91 ***		0 96 ***	0 58 *	
I_{180}	18	0 76 ***	0 62 **	0 72 ***				0 94 ***	0 96 ***		0 47 *	
[illegible]	[illegible]	[illegible]			0 74 ***	0 72 ***	-0 48 *	0 60 **	0 58 *	0 47 *		
[illegible]				0 48 *			-0 51 *					

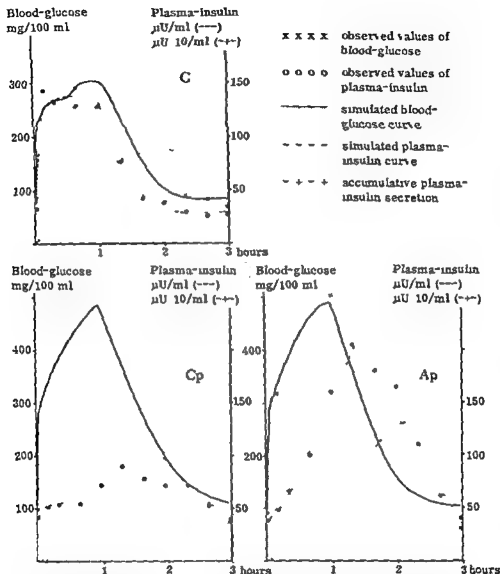


Fig 7 Typical curves of observed and simulated blood-glucose and plasma-insulin and of accumulative insulin secretion from subjects belonging to the control group (C) and the groups with chronic (Cp) and acute (Ap) pancreatitis

TABLE 10 Comparisons of some variables of the GIT between the control group (C_1) and the groups of chronic (Cp_1) and acute (Ap_1) pancreatitis

	C_1 - Cp_1				C_1 - Ap_1				Ap_1 - Cp_1			
	n		difference/ level of significance		n		difference/ level of significance		n		difference/ level of significance	
	C_1	Cp_1			C_1	Ap_1			Ap_1	Cp_1		
log k_{11}	28	20	0 50	**	28	18	0 10		18	20	0 40	*
log k_{12}	28	19	0 32	*	28	18	-0 02		18	19	0 34	*
log b	28	20	0 44	**	28	18	-0 08		18	20	0 52	**
log k_g	28	[illegible]	0 14	*	28	18	0 16	**	18	19	-0 02	
Gu_{60}%	28	[illegible]	17 1	***	[illegible]	18	10 7	***	[illegible]	20	6 4	*
Gu_{180}%	27	19	18 5	***	27	18	4 9	*	18	19	13 6	*
I_{30}%	[illegible]	18	10 6	**	22	18	8 3	**	18	18	2 3	
I_{90}%	22	18	24 2	***	22	18	5 0		18	18	19 2	**

comparisons between the three groups regarding fasting blood-glucose and plasma-insulin values were not found to differ significantly

The group with **chronic pancreatitis** compared to the control group had significantly lower values for all tested variables of the GIT The levels of significance were highest for Gu_{60}%, Gu_{180}% and I_{90}% ($P<0\ 001$) and for log b and log k_{11} ($P < 0\ 01$)

The group of **acute pancreatitis** differed significantly from the control group regarding Gu_{60}% ($P < 0\ 001$ log k_g and I_{30}% ($P < 0\ 01$) All these values were decreased

The group with chronic pancreatitis when compared to that of acute pancreatitis had significantly lower values for log b and I_{90}% ($P < 0\ 01$)

Thus, significant differences of variables of the GIT were found between the three groups (C_1 Cp_1, Ap_1) Gu_{60}% was the only variable showing highly significant difference ($P < 0\ 001$) between the control group versus both groups of chronic and acute pancreatitis Log b and I_{90}% differed the group with chronic from that of acute pancreatitis ($P <0\ 01$)

The Secretin-Pancreozymin Test (SPT)

Distribution of data

The different variables as well as their logarithms have been plotted on normal distribution paper Since logarithmation did not render the distribution more normal the non-transformed values were

TABLE 11 Coefficients of correlation between variables of exocrine pancreatic function and age, body-weight-height body surface area, and lean body mass in the control group

	n	Age	Body weight	Body height	Body surface area	Lean body mass
Secretin stimulation 60 min						
Volume ml	40	−0 13	0 55 ***	0 56 ***	0 [illegible] ***	0 59 ***
Bicarbonate mE	40	−0 20	0 46 ***	0 52 ***	0 50 **	0 50 **
max concn mE/l	30	−0 36 *	−0 25	−0 17	−0 22	−0 24
Amylase AU	40	0 03	0 35 *	0 26	0 32 *	0 32 *
Trypsin mg	2[illegible]	−0 15	0 23	0 40 *	0 30	0 32
Cecekin stimulation 30 min						
Amylase AU	15	0 24	0 37	0 24	0 35	0 37
Trypsin mg	15	0 37	0 09	−0 05	0 03	0 03

used for the statistical analysis All distributions were considered sufficiently normal for statistical calculations Some reservations may be put forward as regards the amounts of amylase and trypsin after cecekin administration and the amount of trypsin after giving secretin This was valid for all groups In addition there was in the group with chronic pancreatitis a tendency towards accumulation of low values for some variables

Control group

Influence of age body measurements and lean body mass on variables of the SPT

The correlation coefficients for the different variables of exocrine pancreatic secretion in relation to age, body measurements and lean body mass are given in Table 11 and the regression of maximal bicarbonate concentration on age in Fig [illegible] A probably significant inverse correlation was found between maximal bicarbonate concentration and age ($P < 0\ 05$) Significant correlations were demonstrated between volume of duodenal juice ($P < 0\ 001$) and amount of bicarbonate during 60 min after secretin injection ($P < 0\ 001$ or $P < 0\ 01$) on one side and body weight and height, body surface area and lean body mass on the other The amount of amylase was probably significantly correlated to some body measurements and lean body mass

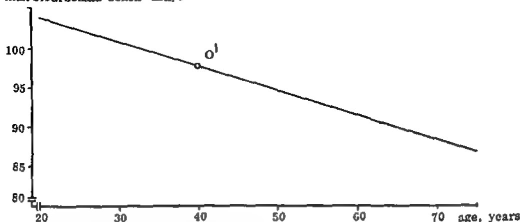


Fig 8 The regression line of maximal bicarbonate concentration on age O[1] is maximal bicarbonate concentration 97 9 mE/l and age 39 8 years The sample regression equation is $y = -0\ 30796x$

during 60 min after giving secretin The correlation coefficients between the body measurements on one hand and amount of amylase after cecckin and amount of trypsin during 60 min after secretin administration on the other, were of the same magnitude as for the above mentioned correlation to amylase but as a rule not significant This may well be explained by the limited number of tests performed

The results imply that measurements of exocrine pancreatic funtion should be *related to body size* Since the regression lines of variables of exocrine pancreatic function on body measurements did not deviate significantly from origo this may be done by dividing the values obtained with the body size parameters No measurement of body size was better correlated to exocrine pancreatic function than weight Therefore, the results have also been presented in values corrected for body weight

Measurements of variables of the SPT

The mean values are presented in Table 12 and the distribution of the values of some variables in Fig 9

The differences between the age groups C_2 (< 40 years), C_3 (40 - 59 years), and C_4 (≥ 60 years) are shown in Table 13 The volume recovered in *60 min after secretin administration* was larger in group C_3 than in C_4 ($P < 0\ 01$) and C_2 ($P < 0\ 05$) The amount of bicarbonate during 60 min after giving secretin was significantly larger in group C_3 than C_4 ($P < 0\ 05$) The highest bicarbonate concentration was seen

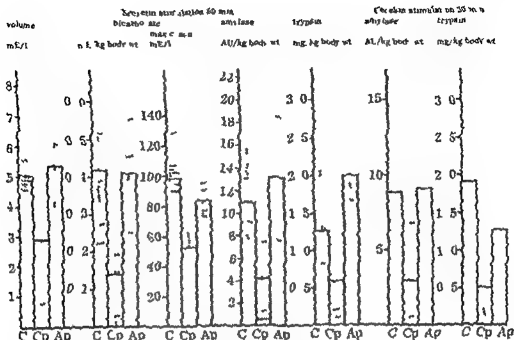


Fig 9 Distributions of some variables of the SPT

in group C_2 and was significantly higher than in group C_3 ($P < 0.05$)

When the above measurements were corrected for body weight the differences between the age groups were less obvious for volume but more so for the amount of bicarbonate both during 60 min after secretin administration

No significant diferences were found between the age and sex groups regarding amounts of amylase and trypsin recovered after cecekin stimulation

Thus, the volume and amount of bicarbonate during 60 min after giving secretin and the maximal bicarbonate concentration decreased with age Though the amylase and trypsin values were numerically higher in the groups of subjects below 60 years compared to that above, the differences were not statistically significant

Sex differences are also presented in Table 13 Males had significantly larger volumes of duodenal juice ($P < 0.01$) than females This difference disappeared when corrected for body weight Thus females and males behaved similarly as regards measurements of the SPT

Correlations between variables of the SPT

Table 14 shows that highly significant correlations ($P < 0.001$) existed firstly between volumes and amounts of bicarbonate, amylase and trypsin collected at different times,

TABLE 12 Measurements of variables of the SPT in the group and subgroups of controls

Groups of subjects	Secretin stimulation, 30 min				
	volume	bicarbonate		amylase	trypsin
	ml ml/kg body wt	mE mE/kg body wt	max concn mE/l	AU AU/kg body wt	mg mg/kg body wt
C_1	184 ± 7	14 2 ± 0 6	98 ± 2	422 ± 31	43 1 ± 5 5
whole group	2 73 ± 0 10	0 21 ± 0 01		6 4 ± 0 5	0 63 ± [illegible]
	n 50	n 50	n 50	n 50	n = [illegible]
C_2	190 ± 8	15 0 ± 0 8	104 ± 3	420 ± 40	37 4 ± 8 1
< 40 years	2 90 ± 0 12	[illegible] 23 ± 0 01		6 6 ± 0 7	0 58 ± 0 15
	n 26	n = 26	n 26	n [illegible]	n 11
C_3	202 ± 17	14 5 ± 1 4	90 ± 4	459 ± [illegible]	50 6 ± 8 2
40 - 59 years	2 80 ± 0 25	0 20 ± 0 02		6 2 ± 1 1	0 72 ± 0 12
	n = 13	n 13	n 13	n = 13	n 12
C_4	148 ± 12	12 0 ± 1 0	92 ± [illegible]	384 ± 43	40 5 ± 12 3
≥ 60 years	2 26 ± 0 15	0 18 ± 0 01		5 9 ± 0 6	0 59 ± 0 15
	n 11	n 11	n 11	n 11	n - 11
C_5	168 ± 10	13 5 ± 0 9	103 ± 4	411 ± 43	42 4 ± 7 6
females	2 88 ± 0 17	0 23 ± 0 01		7 0 ± 0 8	0 74 ± 0 14
	n = 21	n 21	n = 21	n = 21	n 12
C_6	196 ± 10	14 7 ± 0 9	94 ± 3	430 ± 44	43 4 ± 7 5
males	2 63 ± [illegible] 13	0 20 ± 0 01		5 9 ± 0 6	0 57 ± 0 10

secondly between various variables of volumes and amounts of bicarbonate after secretin administration and thirdly between amounts of amylase and trypsin after giving cecekin

On the other hand no or very slight correlations were found between various variables of amylase on one side, and those of volume and bicarbonate on the other Corresponding correlations between trypsin versus volume and bicarbonate were more significant

When the above correlations were tested for values corrected for body weight the same levels of significance were obtained

Secretin stimulation 60 min volume ml ml/kg body wt	bicarbonate mE mE/kg body wt	amylase AU AU/kg body wt	trypsin mg mg/kg body wt	Cecekin stimulation 30 min amylase AU AU/kg body wt	trypsin mg mg/kg body wt
336 ± 14 5 09 ± 0 18 n 40	27 4 ± 1 3 0 42 ± 0 02 n = 40	743 ± 52 11 2 ± 0 8 n 40	84 5 ± 9 8 1 24 ± 0 14 n 25	656 ± 83 8 9 ± 1 1 n = 15	135 8 ± 19 1 1 89 ± 0 26 n 15
131 ± 16 1[illegible] ± 0 23 n 22	27 6 ± 1 6 0 43 ± 0 02 n 22	729 ± 64 11 4 ± 1 1 n 22	86 4 ± 13 8 1 34 ± 0 28 n 7	621 ± 63 8 9 ± 1 0 n 9	113 0 ± 20 3 1 63 ± 0 31 n 9
0 ± 27 77 ± 0 39 n 8	32 5 ± 2 8 0 46 ± 0 04 n 8	821 ± 179 11 1 ± 2 2 n 8	95 0 ± 11 1 1 36 ± 0 18 n 8	708 ± 194 8 9 ± 2 3 n 6	170 1 ± 34 2 2 28 ± 0 42 n 6
± 27 12 ± 0 28 n 10	22 7 ± 2 1 0 33 ± 0 02 n 10	713 ± 68 11 0 ± 1 0 n 10	74 7 ± 21 3 1 10 ± 0 26 n 10		
± 15 ± 0 28 20	24 9 ± 1 6 0 43 ± 0 03 n 20	666 ± 64 11 5 ± 1 1 n 20	73 7 ± 10 [illegible] 1 29 ± 0 20 n 11	421 ± 185 7 3 ± 3 2 n 3	139 2 ± 12 8 2 40 ± 0 15 n 3
± 18 0 22 20	29 8 ± 1 8 0 40 ± 0 02 n 20	820 ± 79 11 0 ± 1 0 n 20	93 0 ± 15 4 1 22 ± 0 19 n 14	714 ± 89 9 3 ± 1 1 n 12	135 0 ± 23 9 1 76 ± 0 31 n 12

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The distributions of the values of some variables are compared to those of the control group in Fig 9

Comparisons between the subgroups Cp_2 (with alcoholism) − Cp_3 (without alcoholism) and Cp_4 (with overt diabetes) − Cp_5 (without overt diabetes) are presented in Table 16

TABLE 13 *Comparisons of* some variables of exocrine pancreatic function between following subgroups of the control group C_2 (< 40 years) C_3 (40 - 59 years) C_4 (≥ 60 years) C_5 (females) and C_6 (males)

	$C_2 - C_3$ n C_2	$C_2 - C_3$ n C_3	$C_2 - C_3$ difference/ level of significance	$C_2 - C_4$ n C_2	$C_2 - C_4$ n C_4	$C_2 - C_4$ difference/ level of significance	$C_3 - C_4$ n C_3	$C_3 - C_4$ n C_4	$C_3 - C_4$ difference/ level of significance	$C_5 - C_6$ n C_5	$C_5 - C_6$ n C_6	$C_5 - C_6$ difference/ level of significance
Secretin stimulation 60 min												
Volume ml	22	8	- 79	22	10	43	8	10	122 **	20	20	- 83 **
ml/kg body wt	[illegible]	8	- 0 62	22	10	0 73	8	10	1 35 *	20	20	- 0 01
Bicarbonate mE	22	8	- 4 9	22	10	4 9	8	10	9 9 *	20	20	- 4 9
mE/kg body wt	22	8	- 0 03	22	10	0 09 **	8	10	0 11	20	20	0 03
max concn mE/l	26	13	13 5 *	26	11	11 8	13	11	- 1 7	21	[illegible]	9
Amylase AU	22	8	- 92	22	10	16	8	10	108	[illegible]	20	-160
AU/kg body wt	22	8	0 3	22	10	0 4	8	10	0 0	[illegible]	20	0 4
Trypsin mg	7	8	- 6 6	7	10	11 7	8	10	20 3	11	14	- 19 3
mg/kg body wt	7	8	- 0 [illegible]	7	10	0 24	8	10	0 26	11	14	0 07

The subgroup of subjects with alcoholism probably had a significantly lower maximal bicarbonate concentration ($P < 0.05$) than the one without alcoholism. Otherwise there were no significant differences in the measurements of the SPT between subjects with and without alcoholism.

Thus, the aetiology of chronic pancreatitis did not appear to influence the exocrine pancreatic function.

The subjects with overt diabetes had significantly smaller amounts of amylase after cecekin administration ($P < 0.01$) and probably significantly smaller amounts of amylase and trypsin during 60 min after giving secretin ($P < 0.05$) than those without overt diabetes.

The differences in exocrine pancreatic function between patients with chronic pancreatitis with and without overt diabetes were more pronounced than between those with and without alcoholism.

Correlations between variables of the SPT

The significant correlations obtained are presented in Tables 17 and 18.

As in the control group highly significant correlations ($P < 0.001$) were found within both groups with pancreatitis between volumes and amounts of bicarbonate and enzymes collected during 30 min versus volumes and amounts collected during 60 min. The same level of significance existed between the correlations between volume and amount of bicarbonate for values corrected and uncorrected for body weight.

In contrast to the control group no significant correlations were found between amylase and trypsin after cecekin administration. This may be due to the small number of subjects.

Comparisons between the control group and the groups of chronic and acute pancreatitis

The results of these studies are presented in Table 19.

In chronic pancreatitis (Cp_1) all measurements of variables of the SPT were significantly decreased ($P < 0.001$ and $P < 0.01$).

The group of acute pancreatitis (Ap_1) showed lower maximal bicarbonate concentrations and larger amounts of trypsin during 60 min after secretin injection than the control group (C_1) $P < 0.001$. The volume during 60 min after giving secretin was larger in Ap_1 than in C_1 ($P < 0.05$). Group Ap_1 in comparison to Cp_1 had significantly larger volumes and amounts of bicarbonate, amylase and trypsin during 60 min after administrating secretin, larger amounts of trypsin after giving cecekin, and higher maximal bicarbonate concentration ($P < 0.001$). The amount of amylase

TABLE 14 Significant correlations between variables of the SPT in the control group

	n	Secretin stimulation 30 min Volume				
		Volume	Bicarbonate	max concn	Amylase	Trypsin
		ml	mE	mE/l	AU	mg
n		50	50	50	50	34
Secretin stimulation 30 min						
Volume ml	50		0 72 ***		0 32 *	0 55 ***
Bicarbonate mE	50	0 72 ***		0 29 *		0 51 **
max concn mE/l	50		0 29 *			
Amylase AU	50	0 32 *				0 45 **
Trypsin mg	34	0 55 ***	0 51 **		0 45 **	
Secretin stimulation 60 min						
Volume ml	40	0 90 ***	0 69 ***			0 59 **
Bicarbonate mE	40	0 69 ***	0 91 ***			0 54[1] **
Amylase AU	40				[illegible] 95 ***	
Trypsin, mg	25	0 55 ***	0 60 **			0 98 ***
Cecekin stimulation 30 min						
Amylase AU	15				0 83 ***	
Trypsin mg	15		0 63 *		0 71 **	0 65 **

[1] n = 25 [2] n = 11

Secretin stimulation 60 min				Cecekin stimulation 30 min	
Volume	Bicarbonate	Amylase	Trypsin	Amylase	Trypsin
ml	mE	AU	mg	AU	mg
40	40	40	25	15	15
0 90 ***	0 69 ***		0 55 ***		
0 69 ***	0 91 ***		0 60 **		0 [illegible] *
		0 95 ***		0 83 ***	0 71 **
0 59 1) **	0 54 1) **		0 95 ***		[illegible] 65 **
	0 79 ***	0 33 *	0 65 ***		0 63 2) *
0 79 ***			0 59 **		0 77 2) **
0 33 *				0 90 2) ***	0 79 2) **
0 65 ***	0 59 **				
		0 90 2) ***			0 77 ***
0 63 2) *	0 77 2) **	0 79 2) **		0 77 ***	

TABLE 15 Measurements of variables of the SPT in the group and subgroups of chronic pancreatitis

Groups of subjects	Secretin stimulation 30 min				
	volume ml ml/kg body wt	bicarbonate mE mE/kg body wt	 max concn mE/l	amylase AU AU/kg body wt	trypsin mg mg/kg body wt
Cp_1	109 ± 13	5 0 ± 0 9	56 ± 5	117 ± 29	24 5 ± 6 1
chronic pancreatitis	1 72 ± 0 19	0 08 ± 0 01		1 7 ± 0 4	0 38 ± 0 09
whole group	n 25	n 24	n 24	n 22	n 19
Cp_2	121 ± 17	4 8 ± 1 0	47 ± 6	115 ± 33	25 4 ± 6 2
chronic pancreatitis	1 94 ± 0 25	0 08 ± 0 01		1 7 ± 0 4	0 41 ± 0 10
with alcoholism	[illegible] 16	n 16	n 16	n = 16	n = 14
Cp_3	89 ± 16	5 4 ± 1 9	73 ± 10	123 ± 65	21 9 ± 17
chronic pancreatitis	1 35 ± [illegible] 24	0 08 ± 0 03		1 7 ± 0 9	0 31 ± 0 22
without alcoholism	n 9	n = 8	n 8	n = 6	n 5
Cp_4	105 ± 20	4 2 ± 1 2	48 ± 8	37 ± 12	103 ± 3 0
chronic pancreatitis	1 70 ± 0 29	0 07 ± 0 02		0 6 ± 0 2	0 18 ± 0 06
with diabetes	n 13	n [illegible]	n = 12	n 11	n 9
Cp_5	115 ± 16	5 8 ± 1 3	64 ± 7	196 ± 46	37 2 ± 9 7
chronic pancreatitis	1 75 ± 0 25	0 09 ± 0 02		2 8 ± 0 6	0 57 ± 0 15
without overt diabetes	n 12	[illegible] 12	[illegible] 12	n 11	n 10
Ap_1	229 ± 14	16 6 ± 1 4	84 ± 3	553 ± 61	88 3 ± 9 1
acute pancreatitis	3 01 ± 0 17	0 22 ± 0 02		7 5 ± 0 9	1 16 ± 0 1
whole group	[illegible] 18	[illegible] 18	n = 18	n 18	n [illegible]

Secretin stimulation, 60 min				Cecekın stimulatıon 30 mı	
volume ml ml/kg body wt	bicarbonate mE mE/kg body wt	amylase AU AU/kg body wt	trypsin mg mg/kg body wt	amylase AU AU/kg body wt	trypsin mg mg/kg body
188 ± 28	9 5 ± 1 9	287 ± 84	38 7 ± 11 5	219 ± 72	34 4 ± 7
2 92 ± 0 40	0 14 ± 0 03	4 2 ± 1 2	0 60 ± 0 19	3 1 ± 0 9	0 51 ± [illegible]
n = 19	n – 19	n = 18	n 13	n = 9	n = 9
213 ± 45	9 1 ± 2 6	212 ± 80	41 1 ± 14 0	206 ± 80	31 6 ± 8
3 37 ± 0 63	0 14 ± 0 04	3 1 ± 1 1	0 67 ± 0 24	3 0 ± 1 1	0 49 ± 0
n = 11	n – 11	n 11	n = 9	n = 7	n = 7
154 ± 24	10 0 ± 2 7	404 ± 175	33 2 ± 22 7	264 ± 225	44 1 ± 24
2 29 ± 0 33	0 14 ± 0 04	5 8 ± 2 7	0 46 ± 0 30	3 3 ± 2 7	0 56 ± 0
n = 8	n = 8	n 7	n = 4	n = 2	n = 2
183 ± 42	8 1 ± 2 6	104 ± 39	16 7 ± 6 6	55 ± 20	22 1 ± 6
2 96 ± 0 58	0 13 ± 0 04	1 6 ± 0 6	0 30 ± 0 13	1 0 ± 0 40	0 38 ± 0
n = 11	n = 11	n = 10	n – 7	n = 5	n = 5
195 ± 36	11 3 ± 2 7	515 ± 150	64 3 ± 19 5	424 ± 74	49 8 ± 12
2 86 ± 0 57	0 17 ± 0 04	7 3 ± 2 2	0 96 ± 0 33	5 7 ± 0 9	[illegible] ± 0
n 8	n = 8	n – 8	n 6	n = 4	n = 4
415 ± 31	31 7 ± 2 9	939 ± 96	150 5 ± 14 4	628 ± 8 7	92 5 ± 9
5 [illegible] ± 0 33	0 41 ± 0 03	12 8 ± 1 5	1 99 ± 0 18	9 2 ± 1 7	1 30 ± [illegible]
n = 18	n = 18	n 18	n 18	n = [illegible]	n = 7

TABLE 16 *Comparisons of variables of exocrine pancreatic function between the subgroups of chronic pancreatitis with and without alcoholism (Cp_2 and Cp_3) and with and without overt diabetes (Cp_4 and Cp_5)*

	Cp_2 - Cp_3			Cp_4 - Cp_5		
	n		difference/ level of significance	n		difference/ level of significance
	Cp_2	Cp_3		Cp_4	Cp_5	
Secretin stimulation, [illegible] min						
Volume ml	11	8	59	11	8	- 12
ml/kg body wt	11	8	1 [illegible]	11	8	0 10
Bicarbonate mE	11	8	- 0 9	11	8	- 3 2
mE/kg body wt	11	8	0 00	11	8	- 0 04
max concn mE/l	16	8	- 25 8 *	12	[illegible]	- 10 3
Amylase AU	11	7	-192	10	8	-411 *
AU/kg body wt	11	7	- 2 6	10	8	- 5 7 *
Trypsin mg	9	4	7 9	7	6	- 47 6 *
mg/kg body wt	9	4	0 20	7	6	- 0 52
Cecekin stim 30 min						
Amylase AU	-	-	-	5	4	-369 **
AU/kg body wt	-	-	-	5	4	- 4 7 **
Trypsin mg	-	-	-	5	4	- 27 7
mg/kg body wt	-	-	-	5	4	- 0 28

after giving cecekin was significantly larger in Ap_1 than in Cp_1 ($P < 0.01$)

The levels of significance were, as a rule, not altered when the amounts were corrected for body weight

As the maximal bicarbonate concentration in the control group was inversely correlated to age all values of this variable in the group with chronic and in that of acute pancreatitis were corrected for the mean age in the control group according to Fig 8 The differences between these corrected values and the mean value in the control group was highly significant as regards the group with chronic ($P < 0.001$) and significant regarding that of acute pancreatitis ($P < 0.01$)

Thus, in general, there were highly significant differences between the variables in C_1 and Cp_1 and between those in Ap_1 and Cp_1 Maximal bicarbonate concentration was the variable which showed the highest significant differences between all three groups (C_1 Cp_1 Ap_1)

TABLE 17 Correlations between variables of the SPT in the group of chronic pancreatitis

	n	Secretin stimulation 30 min volume					Secretin stimulation 60 min volume				Cecekin stimulation 30 min amylase	trypsin
			bicarbonate	max concn	amylase	trypsin		bicarbonate	amylase	trypsin		
		ml	mE	mE/l	AU	mg	ml	mE	AU	mg	AU	mg
n		25	24	24	22	19	19	19	[illegible]	13	9	9
Secretin stimulation 30 min												
volume ml	25		0 86 ***				0 96 ***	0 86 ***				
bicarbonate mE	[illegible]	0 86 ***		0 .5 **	0 50 *	0 67 **	0 82 ***	0 97 ***		0 63 *		
max concn mE/l	21		0 55 **		0 54 **	0 47 *		0 57 *		0 57 *		0 67 *
amylase AU	22		0 50 *	0 54 **		0 55 *		0 53 *	0 93 ***	0 64 *	0 88 **	
trypsin, mg	19		0 67 **	0 47 *	0 55 *			0 57 *		0 96 ***		
Secretin stimulation 60 min												
volume ml	19	0 96 ***	0 92 ***					0 87 ***				
bicarbonate mE	19	0 88 ***	0 97 ***	0 57 *	0 53 *	0 57 *	0 87 ***			0 57 *		
amylase, AU	18				0 93 ***					0 58 *	0 89 **	
trypsin, mg	13		0 63 *	0 57 *	0 64 *	0 9. ***		0 57 *	0 [illegible] *			
Cecekin stimulation, 30 min												
amylase AU	9				0 88 **				0 89 **			(0 54)
trypsin mg	9			0 67 *							(0 54)	

... group of acute pancreatitis

	n	Secretin stimulation 30 min: volume	bicarbonate	bicarbonate max concn	amylase	trypsin	Secretin stimulation 60 min: volume	bicarbonate	amylase	trypsin	Cecekin stimulation 30 min: amylase	trypsin
n		ml	mE	mE/l	AU	mg	ml	mE	AU	mg	AU	mg
		18	18	18	18	18	18	18	18	18	6	7
Secretin stimulation 30 min												
volume, ml	18		0 77 ***			0 64 **	0 96 ***	0 81 ***		0 69 **		
bicarbonate mE	18	0 77 ***		0 50 *	0 55 *	0 58 *	0 83 ***	0 98 ***		0 63 **		
max concn mE/l	18		0 50 *									
amylase AU	18		0 55 *			0 67 **		0 53 *	0 96 ***	0 70 **		
trypsin mg	18	0 64 **	0 58 *		0 67 **		0 58 *	0 55 *	0 64 **	0 90 ***		
Secretin stimulation 60 min												
volume, ml	18	0 96 ***	0 83 ***			0 58 *		0 90 ***		0 67 **		
bicarbonate mE	18	0 81 ***	0 98 ***		0 53 *	0 55 *	0 90 ***			0 63 **		
amylase, AU	18				0 96 ***	0 64 **				0 71 **		
trypsin mg	18	0 69 **	0 63 **		0 70 **	0 96 ***	0 67 **	0 63 **	0 71 **			
Cecekin stimulation 30 min												
amylase, AU	6											
trypsin, mg	7											

TABLE 19 Comparisons of variables of exocrine pancreatic function between the groups C_1 (controls) — Cp_1 (chronic pancreatitis) C_1 — Ap_1 (acute pancreatitis and Ap_1 — Cp_1

	C_1 — Cp_1				C_1 — Ap_1				Ap_1 — Cp_1			
	n		difference/ level of significance		n		difference/ level of significance		n		difference/ level of significance	
	C_1	Cp_1			C_1	Ap_1			Ap_1	Cp_1		
Secretin stimulation 60 min												
Volume ml	40	19	148	***	40	18	- 78	*	18	19	226	***
ml/kg body wt	40	19	2 17	***	40	18	- 0 34	**	18	19	2 51	***
Bicarbonate, mE	40	19	17 9	***	40	19	- 4 3		18	19	22 2	***
mE/kg body wt	40	19	0 27	***	40	[illegible]	- 0 00		18	19	0 27	***
max concn mE/l	50	24	42 2	***	50	18	14 4	***	18	24	28 2	***
Amylase AU	40	18	456	***	40	18	-196		18	[illegible]	652	***
AU/kg body wt	40	[illegible]	7 1	***	40	18	- 1 5		18	18	8 6	***
Trypsin mg	25	13	45 8	**	25	18	- 66 0	***	18	13	111 8	***
mg/kg body wt	25	13	0 64	**	25	18	- 0 74	**	18	13	1 38	***
Cecekin stimulation 30 min												
Amylase AU	15	9	437	***	15	6	28		6	9	409	**
AU/kg body wt					15	6	- 0 3		6	9	6 2	**
Trypsin mg	15	9	101 4	***	15	7	43 3		7	9	58 1	***
mg/kg body wt	15	9	1 38	***	15	7	0 59		7	9	0 79	***

Correlations between Variables of Endocrine and Exocrine Pancreatic Function

Control group

The results of these studies are presented in Table 20 The amount of insulin secreted in 180 min was found to be correlated to the amount of amylase recovered in 60 min after giving secretin ($P < 0\ 01$) The latter variable also had probably significant correlation to log b ($P < 0\ 05$) The coefficients of correlation between parameters of endocrine and exocrine pancreatic function as a rule were higher for the amylase variables than for the other variables of the SPT Correction for body weight of variables of exocrine pancreatic function usually did not change the levels of significance

The exocrine pancreatic function was compared between the six subjects with log $k_{11} < 1\ 800$ and the six with the highest values of log k_{11} ($> 2\ 500$) The group with a low log k_{11} had probably significantly lower amounts of amylase dur-

TABLE 20 Correlations between variables of endocrine and exocrine pancreatic function in the control group

	n	$\log k_{11}$	$\log k_{12}$	log b	I_{30}	I_{60}	I_{180}
n		22	22	22	22	22	22
Secretin stimulation 60 min							
Volume, ml	19	-0 04	0 25	-0 03	-0 14	-0 09	0 11
Bicarbonate, mE	19	-0 05	0 28	0 28	-0 05	-0 12	0 13
max concn, mE/l	22	0 09	0 08	0 03	0 09	-0 02	-0 10
Amylase AU	19	0 24	0 40	0 47 *	0 24	0 38	0 67 **
Trypsin mg	16	0 03	0 40	0 00	-0 18	-0 16	0 20
Cecekin stimulation 30 min							
Amylase AU	7	0 19	0 71	0 50	0 32	0 23	0 61
Trypsin mg	7	-0 24	0 16	-0 05	-0 16	-0 34	0 [illegible]

ing 60 min after giving secretin (P < 0 05) than the other group All other variables of exocrine pancreatic function did not differ between the groups

Thus the group of subjects with low $\log k_{11}$ – which Cerasi and Luft (1967 b) considered to be a criterion on 'prediabetes' – seemed to have another characteristic sign namely low amylase values Furthermore some parameters of endocrine pancreatic function had higher coefficients of correlation to amylase variables than to other variables of exocrine pancreatic function

Groups of chronic and acute pancreatitis

The correlations obtained are presented in Table 21

In the group with chronic pancreatitis there were highly significant or significant correlations between the amount of insulin secreted in 30 60 (P < 0 001) and 180 min (P < 0 01) and $\log k_{11}$ (P < 0 01) on one side and the amount of amylase recovered in 60 min after ad-

TABLE 21 Correlations between variables of endocrine and exocrine pancreatic function in the group with chronic pancreatitis

	n	log k_{11}	log k_{12}	log b	I_{30}	I[illegible]	I_{180}
n		18	18	18	18	18	18
Secretin stimulation 60 min							
Volume, ml	[illegible]	-0 20	0 14	0 09	-0 01	0 02	0 35
Bicarbonate, mE	12	0 20	0 09	0 10	0 13	0 07	0 28
max concn mE/l	15	0 61 *	0 12	0 02	0 24	0 14	0 13
Amylase AU	11	0 75 **	0 59	0 65 *	0 90 ***	0 90 ***	0 78 **
Trypsin, mg	9	0 35	0 18	0 03	0 12	0 06	0 01
Cecekin stimulation 30 min							
Amylase AU	7	0 74	0 44	0 52	0 61	0 61	0 51
Trypsin mg	7	0 65	0 18	0 13	0 33	0 26	0 08

ministrating secretin on the other The correlations were of about the same level of significance with variables of exocrine pancreatic function corrected for body weight

In the group of **acute pancreatitis** there were no significant correlations between variables of endocrine and exocrine pancreatic function

Thus in the group of chronic pancreatitis as in the control group the amount of amylase recovered during 60 min after giving secretin was the exocrine variable with the most significant correlations to various endocrine variables

Comparisons between the three groups regarding correlations between variables of endocrine and exocrine pancreatic function is presented in connection with the discriminant analysis

Other Results

Some of the results of these studies are presented in the Appendix

The **liver function** was studied in both groups with pancreatitis

In the group with chronic pancreatitis there were few patients with manifest liver cirrhosis but several had slight changes in the liver function tests In the group of acute pancreatitis the liver function tests were normal

The excretion of fat in the faeces was determined in subjects with chronic pancreatitis The patients collected faeces for fat determinations at home, what makes the results somewhat unreliable These results are therefore not presented in detail but they were classified as plus when the amount of fat in the faeces exceeded 10 g per day, otherwise as minus

Discriminant analysis

The results of these studies are presented in Tables 22 - 26 The coefficients of discriminant function in some selections are presented in the Appendix

The discriminations were performed with the aid of one or different numbers of variables The following variables were arbitrarily chosen for these separations

1 maximal bicarbonate concentration
2 volume of duodenal juice
3 amount of bicarbonate
4 amount of amylase
5 k-value (intravenous glucose tolerance test)
6 log k_{11}
7 log b
8 glucose uptake during 60 min in per cent of given dose
9 glucose uptake during 180 min in per cent of given dose
10 insulin secreted in 90 min in per cent of total amount secreted
11 insulin secretion in 30 min in IU

The variables 2, 3 and 4 were volumes and amounts collected in 60 min after secretin administration and corrected for body weight

The method applied for discriminant analysis presupposes that all values in the covariance matrix are equal between the three groups Therefore the variances for each of the 11 variables and some coefficients of correlation were compared between the three groups The results were presented on page [illegible]

The different selections of discriminations were carried out firstly with different numbers of variables of pancreatic exocrine function secondly with some of these variables with the addition of the k-value and finally with the above variables combined with parameters of the GIT partly some of those obtained following the first hour and partly some parameters obtained following the whole time of the GIT

There was a tendency towards a better separation between the groups when several exocrine variables were applied When endocrine parameters were added the separations were still better The

results of some of these studies are presented in Table 22

In separating the control group and the group with chronic pancreatitis the probabilities of misclassifying a subject decreased from 0 24 to 0 04 when several variables were applied instead of one The corresponding figures for the separations between the control group and the group of acute pancreatitis were 0 31 and 0 15, respectively

The classification of every subject was compared between selection 1 on one side and all other selections in turn on the other according to a tabular form (Table 23) The results of these calculations are presented in Table 24 There was a tendency towards fewer misclassifications with several variables but as a rule no significant difference was obtained probably because of the small number of subjects

Thus discriminant analysis improved the separations between the groups when several variables were applied

The above discriminations were performed on the same subjects in all selections in order to test the effect of additional variables Thereby the maximal number of subjects could not be included in several selections

In an attempt to perform the discriminations with as many subjects as possible and with exclusion of the parameters of the GIT which may not generally be available the following variables were applied (selection 5) firstly maximal bicarbonate concentration (1), secondly volume (12) amounts of bicarbonate (13) amylase (14) and trypsin (15) all collected during 30 min after giving secretin and corrected for body weight, and finally k-value (5) All variances of the variables were compared between the groups according to Bartlett The variances of the amounts of amylase (14) were the only that differed between the groups ($P < 0.05$)

The coefficients of correlation between variables in the groups with these subjects were not included in the computer program It is reasonable to presume that the relations between the coefficients of correlation in the different groups were about the same as those tested above

Thus it was reasonable to presume that the prerequisites for discriminant analysis were fulfilled also in this selection

The results of these studies are presented in Tables 22 24 - 25

The probability of misclassification between the control group and the group with chronic pancreatitis was found to be 0 06

The accuracy of some classifications was also estimated according to the equations 8 and 9 on page 29 The mean of the discriminant function in every

group in selections 4 and 5 was in turn substituted for d_o The results are presented in Table 26 The probabilities obtained were at the lowest about [illegible] 0002

Thus, the accuracy of the discriminations performed was rather good

TABLE 22 Borderline values (V) between the distributions from two groups of one variable (selection 1) and of the discriminant function (selection 2 - 5) and probabilities (P) of misclassifying subjects in the different selections The number of subjects in the different selections and groups were selection 1 - 4, the control group (C) n_C ~ [illegible] the group with chronic (Cp) and acute (Ap) pancreatitis n_{Cp} = 11 and n_{Ap} = 13 respectively, selection 5 n_C - 34 n_{Cp} = 19 and n_{Ap} = 18

Selection, no	Variables no	C - Cp V	C - Cp P	C - Ap V	C - Ap P	Ap - Cp V	Ap - Cp P
1	1	83 7	0 24	89 5	0 31	76 8	0 [illegible]
2	1-5	8 1032	0 12	10 1520	0 25	8 8726	0 [illegible]
3	1-4, 6, 8 11	14 2156	0 09	10 6500	0 15	11 0088	0 12
4	1-11	12 2780	0 04	10 3140	0 15	1 7710	0 [illegible]
5	1, 5, 12-15	10 2612	0 06	4 8850	0 23	11 0495	0 09

TABLE 23 Tabular form for comparing misclassifications in the different selections

Classifications according to selection no 1	selection no 2 (and 3 - 5)	Number of subjects
right	right	
wrong	wrong	
right	wrong	n_1
wrong	right	n_2

TABLE 24 Comparisons between misclassifications with selection 1 and selections 2 – 5 in discrimination between the control group (C) and the groups with chronic (Cp) and acute (Ap) pancreatitis In Table 23 n_1 and n_2 are explained

Selection no 1												Selection
C – Cp				C – Ap				Ap – Cp				
[illegible]		Cp		C		Ap		Ap		Cp		
n_1	n_2	n_1	n_2	n_1	n_2	n_1	n_2	n_1	n_2	n_1	n_2	no
1	0	1	3	2	2	2	3	1	2	1	4	2
1	0	0	4	1	2	1	4	[illegible]	3	1	4	3
0	0	0	3	1	2	1	4	1	2	1	[illegible]	4
1	2	0	3	5	5	0	7	1	1	[illegible]	4	5

TABLE 25 Observed number (n) of misclassifications per total number (N) of subjects in each group and in per cent $\left(\frac{n}{N} \quad 100\right)$

Selections	C – Cp				C – Ap				Ap – Cp			
	C		Cp		C		Ap		Ap		Cp	
no	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
4	1/19	5 3	1/11	9 1	2/19	10 5	1/13	7 7	2/13	15 4	1/11	9 1
5	3/34	8 8	1/19	5 3	8/34	23 5	1/18	5 6	1/18	5 6	1/19	5 3

TABLE 26 Probabilities (P(C) P(Cp) P(Ap)) for subjects belonging to one of the groups (see Fig 4) to obtain a value of the discriminant function equal to or more deviating than the mean of the other group ($\bar{x}_C$ $\bar{x}_{Cp}$ $\bar{x}_{Ap}$)

Selection no	P(C $\bar{x}_{Cp}$)	P(Cp $\bar{x}_C$)	P(C $\bar{x}_{Ap}$)	P(Ap $\bar{x}_C$)	P(Cp $\bar{x}_{Ap}$)	P(Ap $\bar{x}_{Cp}$)
4	0 0002	< 0 0001	0 0150	0 0217	0 0015	0 0009
5	[illegible] 0006	0 0014	0 0594	0 0853	0 0037	0 0032

DISCUSSION

In the present investigation the recently developed glucose infusion test GIT was used in addition to the common intravenous glucose tolerance test for evaluating pancreatic endocrine function. In this study "endocrine function" is confined to the secretion of insulin. The GIT implies calculation of the effect of sustained glucose infusion both on insulin release and on glucose uptake by the tissues. The release of insulin was evaluated both in terms of the relationship between plasma-insulin and its stimulus the blood-glucose concentrations and in terms of the insulin secretion rate during the GIT. Glucose uptake represented in this study the net result of the disappearance of glucose from the blood where glucose loss through the kidneys was taken into account but not the decrease in glucose output by the liver during hyperglycemia.

It was shown that patients in the group with chronic pancreatitis as well as those who had had only one attack of acute pancreatitis showed changes in the glucose and insulin parameters of the GIT.

To sum up the results, among 27 patients with chronic pancreatitis there were 14 subjects with overt diabetes, six with decreased and seven with normal intravenous glucose tolerance. It was shown that both the initial insulin release and the glucose uptake were decreased in the patients with diabetes or decreased intravenous glucose tolerance. The distribution of log k_{11} values in the subjects with diabetes was similar to the corresponding distribution in diabetic subjects reported by Cerasi and Luft (1967 a). It is of special interest that low parameter values of the GIT also occurred in patients with normal intravenous glucose tolerance. Most of these subjects had log k_{11} values around 1.8, the arbitrarily chosen border value between high and low responses (Cerasi and Luft 1967 a). Only one subject in the whole group had a log k_{11} value higher than 2.2.

In this connection it should be remem-

bered that Cerasi and Luft (1967 a) demonstrated low log k_{i1} values in about 15 - 20 per cent in a group of 85 healthy subjects

The whole group of subjects with chronic pancreatitis showed with all evidence available decreased insulin secretion and glucose uptake following glucose infusion It should be noted that the subjects with chronic pancreatitis in this study were not selected among those with an advanced stage of the disease but represented an average encountered at a non-specialized clinic

The results obtained were compared with those obtained by similar studies of healthy subjects The importance of such a comparison becomes evident from the finding that glucose uptake during the first hour of the GIT decreased with increasing age The author found similar results when recalculating the results reported by Cerasi and Luft (1967 a) In spite of this the observed values have not been corrected for age The reason is that the mean age of the control subjects constituting the basis for the above calculations was 49 6 years i e nearly the same as the mean age in the different groups with pancreatitis

Several authors have called attention to the increased frequency of diabetes and decreased glucose tolerance in subjects with chronic pancreatitis The present investigation confirms these observations However earlier studies on insulin secretion in subjects with chronic pancreatitis are unsatisfactory Keller et al (1965) in patients with diabetes due to chronic pancreatitis found normal or raised levels of circulating serum insulin and insulin-like activity This statement was based on isolated serum insulin values where blood-glucose concentrations were not taken into account Furthermore the material of Keller et al included patients who already had been given insulin This renders radioimmunoassays of insulin unreliable because of the occurrence of insulin antibodies

Peters et al (1966) reported that five patients with chronic pancreatitis — all of whom had steatorrhea and mild diabetes and four pancreatic calcification — showed inadequate production of insulin These subjects evidently were in an advanced stage of the disease Furthermore even in this small group blood-glucose concentrations were not taken into account which makes the results of the insulin determinations less valid

As to **acute pancreatitis** changes in the concentrations of blood-glucose and plasma-insulin have previously been reported in the acute stage of the disease (Tsukiyama 1963 Rudyi and Caplinsky 1965) whereas the glucose-insulin interrelationship has not

been studied previously in subjects who have recovered from a single attack of acute pancreatitis

In the present study, in patients with a history of one attack of acute pancreatitis some parameter values of the GIT were found to differ both from those of the control group and those of the group with chronic pancreatitis The main finding was that the glucose uptake during the first hour was lower than in the control group but higher than in the group with chronic pancreatitis

The results of the GIT in the two groups with chronic pancreatitis might be the basis for a postulate of the following course of events regarding glucose-insulin relationship in pancreatitis of increasing severity

In subjects following a single attack of acute pancreatitis the most significant change of the GIT parameters would be a decreased initial release of insulin Indeed the subjects who had had acute pancreatitis often showed a decreased release of insulin during the first 30 min in per cent of the amount secreted during the whole test This decrease would be expected to be accompanied by a lowering of the initial glucose uptake — which infact was found in these subjects As a consequence, blood-glucose concentration would rise leading to increased stimulation for insulin release during a later phase of the GIT

In subjects with more advanced degrees of pancreatitis more pronounced changes in the values of the GIT would appear As already stated the insulin release was markedly decreased in the present group with chronic pancreatitis This would be followed by higher blood-glucose levels which in turn, would stimulate the β-cells to prolonged insulin secretion The level of plasma-insulin then would depend on the capacity of the β-cells of the damaged pancreas to respond to this stimulation In the present group the prolonged secretion of decreased amounts of insulin was marked The glucose uptake as expected was decreased during the first as well as the remaining two hours of the GIT

Thus pancreatitis seems to influence insulin secretion It is then reasonable to ask whether pancreatitis plays a rôle in determining the incidence of diabetes in the general population

In groups with diabetic patients the frequency of subjects where pancreatitis was considered an aetiological factor was calculated as 0 30 (Sprague 1947) and 0 38 per cent (Bell 1958) From Nigeria — where pancreatic disease due to malnutrition is very common — Kinnear (1963) reported the occurrence of pancreatic calcification in more than 13 per cent of diabetic patients

Furthermore, pancreatitis often remains undiagnosed Reviewing records from 35 500 consecutive autopsies Edmundsen et al (1949) found 62 instances with chronic pancreatitis Not a single one had been diagnosed or suspected during life In addition undiagnosed acute pancreatitis severe enough to cause death has been found at autopsy (Wagermark et al 1966) Since chronic pancreatitis and even severe acute pancreatitis can remain undetected undiagnosed pancreatitis of minor degree is probably much more common than hitherto assumed In some of these subjects pancreatitis may alter the insulin secretion and thereby predispose to diabetes

Biliary tract disease, the most common aetiological factor in pancreatitis was often found in diabetic patients (Twiss and Carter 1952) These subjects might have had pancreatitis resulting in diabetes Furthermore fibrosis – undistinguishable *from that seen in some* subjects with pancreatitis – was found in the pancreas of diabetic patients (Warren et al 1966) However, these studies do not permit any conclusion regarding the significance of biliary tract disease in the pathogenesis of diabetes

In conclusion it may be stated that established pancreatitis probably is an unusual aetiological factor in diabetes mellitus Undiagnosed pancreatitis may occur more frequently but is probably of less importance in this connection than hereditary factors

In order to relate the capacity of insulin secretion to the degree of damage to the pancreas e x o c r i n e p a n c r e a t i c f u n c t i o n had to be evaluated This was accomplished by studying the response of the exocrine pancreas to high doses of secretin and in several subjects also to pancreozymin stimulation

In the group with c h r o n i c p a n c r e a t i t i s all variables of exocrine pancreatic function were found to be significantly lower than in the control group Most other authors have found less significant differences (Dreiling et al 1964) It is probable that the higher dose of secretin given in this study accounts for the more significant differences

In the group of a c u t e p a n c r e a t i t i s some measurements of exocrine *pancreatic function were found to be* lower and some unexpectedly higher than in the control group Maximal bicarbonate concentration was lower in subjects who had had acute pancreatitis than in the control group and this parameter was the best one for separating all three groups of subjects Other variables, such as the volume of recovered duodenal juice and its amylase and trypsin contents after secretin administration were higher in the group of

acute pancreatitis than in the control group

There may be several explanations for the high values of some variables of exocrine pancreatic function after acute pancreatitis Most of these patients had been cholecystectomized Thus, the bile could pass freely into the duodenum during the test and increase the volume of the duodenal juice According to Lagerlof (1968) 50 ml of bile containing 3 5 mE of bicarbonate may be added to the duodenal juice during a standard one hour secretin test (Lagerlof 1942) thus lowering the maximal bicarbonate concentration as was found in these patients

The question then arises whether the changes of exocrine pancreatic function in the group of acute pancreatitis merely reflected a condition generally found in patients who have undergone cholecystectomy and not changes in exocrine pancreatic function Further studies on patients after acute pancreatitis with intact function of the gall-bladder would help to solve this problem The changes of insulin secretion in the group of acute pancreatitis are in favour of persisting pancreatic damage

The results of exocrine pancreatic function tests in the two groups with pancreatitis were compared with the corresponding results in a control group in which different age groups were well represented Some of the results in the control group revealed new aspects of pancreatic function Thus several measurements of exocrine pancreatic function were found to be significantly correlated to different body measurements The regression lines for different indices of exocrine pancreatic function in relation to body weight were found to pass close to the origin Therefore a correction for body weight is considered justified

Furthermore some variables of exocrine pancreatic function in the control group tended to decrease with increasing age This agrees with the findings of histological changes in the pancreas of older people (Lidin and Scheidegger 1941 Blumenthal and Probstein 1960 Walters 1964) Rosenberg et al (1966) on the other hand found no effect of age on pancreatic secretion of fluid and bicarbonate This might be due to lower doses of secretin given in their study and also to differences in the age distribution between their subjects and those in the present study The mean age in their older age groups was for females 58 8 and for males 62 8 years, compared to 71 4 and 73 8 years respectively, in this study

The present investigation demonstrated a tendency towards lower values of some variables of exocrine pan-

creatic function in older people but further studies are necessary to make clear to what degree age influences this function The differences between the age groups were significant only regarding some variables, and never showed high levels of significance Measurements of exocrine pancreatic function were therefore only exceptionally corrected for age in the present study

In conclusion all variables of exocrine pancreatic function were found to be lowered in the group with chronic pancreatitis In the group of acute pancreatitis some variables showed decreased and other increased values In the control group exocrine pancreatic variables were found to be correlated to some body measurements It was shown that correcting the values for body weight by division was justified Also some of the variables tended to decrease with increasing age

The quantitative relations between endocrine and exocrine pancreatic functions have previously been inadequately studied owing to unreliable methods

In the present investigation it was shown that some parameters of the GIT were correlated to the amount of amylase recovered during 60 min after secretin administration These were the findings in the group with chronic pancreatitis and to some extent, in the control group In subjects with chronic pancreatitis combined with overt diabetes the amylase content was significantly lower than in those without diabetes Furthermore in the control group the six subjects with $\log k_{i1} < 1$ also presented lower amylase values than the six with the highest $\log k_{i1}$ values On the other hand, in subjects with one attack of acute pancreatitis in the case history no significant correlation was obtained between insulin secretion and amylase content in the duodenal juice

There are reports trying to elucidate the structural relations between the pancreatic islets and the exocrine pancreas These may contribute to the understanding of the functional relations between these two parts of the gland Both the endocrine and exocrine parts of the pancreas are supposed to regenerate from the same ductular epithelium (Tiscornia and Dreiling 1966) The vascular supply to the two parts of the pancreas is such that the products of the pancreatic islets will reach the exocrine pancreas in high concentrations (Thiel 1954) Furthermore zymogen granules in the exocrine cells were found to be most abundant near the pancreatic islets (Jarotzky 1899 Burkl 1949 Hellman et al 1962 Wallgren and Hellman 1962) and the granules enhanced following oral antidiabetica (Ferner 1958) The excretory pancreatic response to tolbutamide was considered

to be better in normals than in diabetics (Knick et al 1964)

The results of the present study do not elucidate whether the secretion of other pancreatic enzymes is affected in a similar way as amylase One report in the literature could indicate that insulin in alloxan diabetic rats enhances the production of amylase but not that of chymotrypsin which like trypsin is a protease (Ben Abdeljlil et al 1965)

In conclusion significant correlations were found between various indices of endocrine pancreatic function and of amylase secretion These correlations were most significant in the group with *chronic pancreatitis* but *also noticeable* in the control group

The information presented here on endocrine and exocrine pancreatic function has been *subjected to* d i s c r i m i n a n t a n a l y s i s in an attempt to improve the differentiation of the two kinds of pancreatic disorder from the normal state

Discriminant analysis implies that the capacity of each variable in separating two groups is estimated, giving to the variable a coefficient The sum of the products of the observed values and the coefficients gives to each subject a value of the discriminant function These values are normally distributed for each of the two tested groups provided that the distributions of the observed values in each group are normal Different combinations of indices (selections) were tested in order to separate these distributions as much as possible giving a borderline value with least probability of misclassification Furthermore, the accuracy of the classifications could be estimated for every subject

The results showed that the ability to classify a subject to the right group applying the parameter values increased when more indices of exocrine pancreatic function were applied If, in *addition indices of* pancreatic endocrine function were adopted the confidence of the classification increased further The classifications were evaluated by comparing the numbers of *misclassifications* and by calculating the probabilities of misclassification in the different selections

For the separation of patients with chronic pancreatitis from normals the *probability of misclassification* was markedly decreased when several indices were taken into account instead of one

In conclusion discriminant analysis *was found to be* a useful tool in separating subjects with pancreatitis from normals

SUMMARY

The aim of the present study was to investigate endocrine and exocrine pancreatic functions and their relationship in subjects with chronic pancreatitis and in those having had a single attack of acute pancreatitis. Furthermore discriminant analysis was applied in order to separate the groups with pancreatitis from each other and from the control group

In the group with chronic pancreatitis overt diabetes occurred in 14 out of 27 subjects. Seven of the diabetics were on insulin treatment and therefore not tested with the GIT Six of the remaining seven subjects with diabetes had a log k_{i1} value less than 1 8 — the arbitrarily chosen border between low and high responses Out of the 13 subjects without manifest diabetes six had a decresed intravenous glucose tolerance One of the 13 patients had a log k_{i1} value of [illegible] 6 but all the others had values around 1 8 (range < 1 0 – [illegible] 1) Thus most of the [illegible] subjects with chronic pancreatitis tested with the GIT demonstrated a low initial insulin response

Out of 23 subjects with acute pancreatitis in the case history none had manifest diabetes but four had a decreased glucose tolerance Eight subjects in this group had values of log k_{i1} < 1 8 and another eight subjects had values between 1 8 and 2 2, but four had log k_{i1} values > 2 6 Thus about 16 out of the 23 subjects in this group seemed to have a rather low initial insulin response

Exocrine pancreatic function was found to be markedly decreased in the group with chronic pancreatitis In the group with acute pancreatitis in the case history some of the variables of exocrine pancreatic function were decreased and some were increased Maximal bicarbonate concentration differed with high level of significance the two groups with pancreatitis from each other and from the control group

In the control group some parameters

of both endocrine and exocrine pancreatic functions were found to be correlated to age and/or body measurements. Correction for body weight of values of some pancreatic exocrine variables by division was found to be justified

Some parameters of endocrine pancreatic function showed correlations to some variables of amylase secretion This was most pronounced in the group with chronic pancreatitis, but also found in the control group Control subjects with low and high insulin response (low and high log k_{i1} values) were inclined to have low and high values of amylase secretion respectively

The ability to separate the three groups of subjects increased when discriminant analysis was applied The probability of misclassifying subjects with chronic pancreatitis from normals decreased from 0 24 to 0 04 by applying several variables instead of one

The accuracy of the classifications was also estimated by calculating the probability for a subject, belonging to one of the two groups in discrimination to obtain a value of the discriminant function equal to or deviating more than the mean value in the other group These probabilities were at the lowest about 0 0002 and 0 02, respectively in the discriminations of subjects with chronic pancreatitis and those having had acute pancreatitis versus normals

Thus discriminant analysis improved the separations between the different groups

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REFERENCES

When discrepancies occur between references in the text and in this list the latter is to be considered the correct version

Ågren G , Lagerlof H and Berglund H Acta med scand 90 224 1936

Anderson T W An Introduction to Multivariate Statistical Analysis John Wiley & Sons Inc New York 1958

Bank S Marks I N Moshal M G Efron G and Silber R S Afr med J 19 Oct 1061 1963

Banting F G and Best C H J Lab clin Med 7 464 1921-22

Baron J H Perrier C V Janowitz H D and Dreiling D A Amer J Physiol 204 251, 1963

Bartelheimer H Dtsch med Wschr 78 993 1953

Bayliss W M and Starling E H J Physiol (Lond) 28 325, 1902

Bell E T Surgery 43 527, 1958

Ben Abdeljlil A Palla J C and Desnuelle P Biochem biophys Res Commun 18 71 1965

Bernard Cl Memoire sur le pancreas et sur le rôle du suc pancréatique dans les phenomènes digestifs, particulièrement dans la digestion des matières grasses neutres J -B Baillière Paris 1856

Bernhard Fr Dtsch Z Chir 212 209, 1928

Blumenthal H T and Probstein J H Arch Surg 81 396, 1960

Brocq P and Varangot J J Chir (Paris) 49 177, 1937

Brohult J Scand J clin Lab Invest 19 67 1967

Burki W Wien klin Wschr 61 264, 1949

Burton P Evans D G Harper A A , Howat H T Oleesky S Scott J E and Varley H Gut 1 111, 1960

Calzavara D Zbl Chir 51 1405, 1924

Cawley T London med. J 9 286, 1788 (cited by Lazarus and Volk 1962)

Cerasi E Acta endocr (Kbh) 55 163, 1967 a

Cerasi E Plasma Insulin Response to Glucose Infusion in Healthy Subjects Patients with Diabetes Mellitus and Subjects with Genetic Prediabetes Thesis, Stockholm 1967 b

Cerasi E and Luft R Lancet ii 1359, 1963

Cerasi E and Luft R Acta endocr (Kbh) 55 278, 1967 a

Cerasi E and Luft R Acta endocr (Kbh) 55 330, 1967b

Cerasi E and Luft R Diabetes 16 615, 1967 c

Chiray M Salmon A -R and Mercier A Bull Soc méd Hôp Paris 50 1417, 1926

Cole W H Amer J Surg 80 245, 1938

Creutzfeldt W Verh dtsch Ges inn Med 70 Kongr 1964 p 781

Diamond J S Siegel S A , Gall M B and Karlen S Amer J dig Dis 6 866 1939

Documenta Geigy Wissenschaftliche Tabellen J R Geigy S A , Basle 1960

Dreiling D A Gastroenterology 24 540 1953

Dreiling D A and Hollander F Gastroenterology 11 714, 1948

Dreiling D A and Janowitz H D Gastroenterology 30 382 1956

Dreiling D A Janowitz H D and Perrier C V Pancreatic Inflammatory Disease A Physiologic Approach Hoeber Medical Division New York 1964

Du Bois D and Du Bois E F Arch intern Med 17 863 1916

Dubois J -P L insuffisance pancreatique externe Thesis no 2090 Karger Basle 1952

Duncan P R , Harper A A , Howat H T Oleesky S and Varley H J Physiol (Lond) 111 63 1950

Edmondson H A Bullock W K and Mehl J W Amer J Path 25 1227, 1949

Erlanger B F Kokowsky N and Cohen W Arch Biochem 95 271, 1961

Feldman M Dreiling D A , Paulino-Netto A Schaffner F and Janowitz H D Amer J Physiol 205 878 1963

Ferner H Dtsch med Wschr 83 1468, 1958

Gaebler O H Amer J clin Path 15 452, 1945

Hald A Statistical Theory with Engineering Application John Wiley & Sons New York 1960

Hales C N and Randle P J Biochem J 88 137 1963

Hammarsten E Ågren G , Hammarsten H and Wilander O Biochem Z 264 275, 1933

Hansky J , Tiscornia O M Dreiling D A and Janowitz H D Amer J Physiol 206 351 1964

Hansson K , Lundh G and Stenram U Acta chir scand 121 274, 1961

Harley G Trans path Soc Lond 13 118 1862

Harper A A and Raper H S J Physiol (Lond) 102 115, 1943

Hellman B Wallgren A and Peters-

son B Acta endocr (Kbh) 39 465, 1962

Henning N , Heinkel K and Schon H Pathogenese, Diagnostik Klinik und Therapie der Erkrankungen des exokrinen Pankreas Europaisches Pankreas-Symposion 24 5 bis 26 5 1963 in Erlangen F K Schattauer-Verlag, Stuttgart 1964

Howat H T Pancreatitis In Modern Trends in Gastro-Enterology p 766 Ed F A Jones Butterworth & Co Ltd London 1952

Hume R J clin Path 19 389 1966

Ikkos D and Luft R Acta endocr (Kbh) 25 312 1957

Jarotzky A J Virchows Arch path Anat 156 438 1899

Jendrassik L and Grof P Biochem Z 297 81 1938

Jorpes J E and Mutt V Acta chem scand 15 1790 1961

Karam J H , Grodsky G M and Forsham P H Diabetes 12 197 1963

Keller P Bank S , Jackson W P U , Marks I N and O Reilly I G Lancet ii 1211 1965

Kinnear T W G E Afr med J 40 288 1963

Knick B Lange H -J Baier H Jäger H , Jockel H and Haas-Scheuren G Klin Wschr 42 34 1964

Lagerlof H O Pancreatic Function and Pancreatic Disease Studied by Means of Secretin Acta med scand Suppl 128 1942

Lagerlof H O Pancreatic Secretion Pathophysiology In Handbook of Physiology - Alimentary Canal II, p 1027 Ed C F Code The Williams & Wilkins Co Baltimore 1968 In press

Lagerlof H O , Schutz H B and Holmer S Gastroenterology 52 67 1967

Laguesse E C R Soc Biol (Paris) 45 819, 1893

Langerhans P Beitrage zur Mikroskopischen Anatomie der Bauchspeicheldruse G Lange Berlin 1869 (cited by Lazarus and Volk 1962)

Lataste J and Neveux J -Y Presse med 72 389 1964

Lazarus S S and Volk B W The Pancreas in Human and Experimental Diabetes *Grune & Stratton* New York 1962

Lidin M and Scheidegger S Klin Wschr 20 690 1941

Lundbaek K Brit med J June 2 *1507 1962*

MacCallum W G Bull Johns Hopk Hosp 20 265, 1909

Marks I N and Tompsett S L Quart J Med 27 431 1958

Marks I N Banks S and Jackson W P U Excerpta med int Congr Ser (Amst) 74 128 1964 (cited by Peters et al 1966)

McGillivray D C , Stordy S N and Bogoch A Canad med Ass J 94 1261 1966

von Mering J and Minkowski O Arch exp Path Pharmak 26 371, 1889

Mikkelsen O Acta chir scand 75 373 1934

Ohlsén P Report at the 6th Congress of the International Diabetes Federation Stockholm 1967

Penau H and Simonnet H Bull Soc Chim biol (Paris) 7 17, 1925

Peters N, Dick A P, Hales C N, Orrell D H and Sarner M Gut 7 277, 1966

Plessier J, Lemonnier F Rubman A, Troupel S, Duhamel F, Plessier B and Legousse S Rev int Hepat 13 491 1963

Rick W Zur Physiologie und Pathologie der Enzymsekretion des Pankreas *Habilitationsschrift* Giesen 1964

Rosenberg I R Friedland N Janowitz H D and Dreiling D A Gastroenterology 50 191, 1966

Rudyi R V and Chaplinsky V V *Klin Med (Mosk)* 43 77, 1965

Sarles H Pancreatitis Symposium Marseilles April 25 and 26 1963 Ed H Sarles S Karger AG, Basle 1965

Schütz H B and Reizenstein P Amer J dig Dis 8 904, 1963

Seldinger S I Acta radiol (Stockh) 39 368 1953

Shumacker H B Ann Surg 112 177, 1940

Snedecor G W Statistical Methods Applied to Experiments in Agriculture and Biology The Iowa State University Press Ames 1964

Sprague R G Proc Mayo Clin 22 553, 1947

Ssobolew L W Virchows Arch path Anat 168 91, 1902

Sun D C H Gastroenterology 45 203, 1963

Thiel A Z Zellforsch 39 339, 1954

Tiscornia O M and Dreiling D A Gastroenterology 51 267, 1966

Tsukiyama T In IInd World Congress of Gastroenterology Munich 1962, IV p 123 Ed H Bartelheimer and H Lagerlof Karger Basle 1963

Twiss J R and Carter R F Amer J med Sci 224 263, 1952

Véghelyi P V and Kemeny T T In Ciba Foundation Symposium on the Exocrine Pancreas Normal and Abnormal Functions p 329 Ed A V S de Reuck and M P Cameron J and A Churchill Ltd, London 1962

Wågermark J Moberg A and Svenhamn K XXIII Medicinska Riksstamman Stockholm 1966

Wallgren A and Hellman B Acta anat (Basel) 48 137, 1962

Walters M N -I Amer J Path 44 973 1964

Wang C C, Wang K J and Grossman M I Amer J Physiol 160 115, 1950

Warren S, LeCompte P M and Legg M A The Pathology of Diabetes Mellitus Lea & Febiger Philadelphia 1966

Wenger J and Raskin H F Gastroenterology 34 1009, 1958

Wildegans H Chirurg 8 597, 1936

Zackrisson L and Reizenstein P Personal communication

APPENDIX

The tables presented in the Appendix are listed below Subjects no 1 – 27 and 28 – 50 refer to the groups with chronic and acute pancreatitis respectively and subjects no 51 – 100 refer to the control group

Primary data on insulin secretion rate are available on request

TABLE 27 General data on subjects with chronic pancreatitis

TABLE 28 General data on subjects having had one attack of acute pancreatitis

TABLE 29 Laboratory data The following normal values were applied calculated *in amounts per 100 ml serum*

bilirubin	< 1 2 mg
total protein	6 – 8 g
albumin	3 6 – 4 6 g
α_1 globulin	0 1 – 0 3 g
α_2 globulin	0 4 – 0 7 g
β globulin	0 7 – 1 9 g
γ globulin	0 8 – 1 5 g

Normal values were for alkaline phosphatase 2 – [illegible] Buch-Buch units and for GOT and GPT 10 – 35 Karmen units

Normal values for the bromsulphthalein retention test were

retention after 25 min < 15 per cent

retention after 45 min < 5 per cent

Intravenous glucose tolerance test was considered normal for k-values ≥ 0 95

TABLE 30 Coefficients of the discriminant function in some selections

TABLE 31 Primary data on the GIT

TABLE 32 Parameter values of the GIT (log k_{i1} log k_{i2} and log b stand for log k_{i1} 10^3 log k_{i2} 10^2 and log [illegible] 10^2 respectively)

TABLE 33 Primary data on the SPT (subject no 47 was gastroectomized and therefore the results of the SPT in this patient were excluded from statistical calculations)

TABLE 27

Subjects			Age in years at					Specifications on pancreatitis													Remarks
								aetiology					symptoms and signs					number of attacks			
no	initials	sex	body wt kg	diagnosis of pancreatitis	diagnosis of diabetes	last attack of pancreatitis	present examination	alcoholism	prim biliary tract disease	second biliary tract disease	other possible cause	unknown	pancreatic calcification	steatorrhea	diarrhoea	pain	borborygmus	3 - 5	> 5	silent type	Heredity for diabetes	
1	R L	F	49	34	37	42	42	-	+	-	-	-	+	+	+	+	+	-	+	-	-	op cholelithiasis and pancreatic cyst insulin treatment pancreatic *enzyme* treatment
2	B J	M	50	35	35	36	38	+	-	-	-	-	-	-	-	-	-	+	-	-	-	insulin treatment
3	D R	M	75	40	44	44	44	+	-	-	-	-	-	-	-	+	+	+	-	-	+	
4	E O	M	65	45	43	-	45	+	-	-	-	-	+	+	+	-	+	-	-	+	+	insulin treatment
5	G H	M	66	50	51	51	51	-	-	-	-	+	-	-	-	+	+	-	+	-	-	op papillotomy tolbutamide treatment myocardial infarction
6	K Å	M	83	41	42	-	51	+	-	-	-	-	+	+	+	-	+	-	-	+	-	pancreatic enzyme treatment insulin treatment
7	G V O	M	[illegible]	45	48	47	55	+	-	-	-	-	+	+	+	-	+	-	+	-	+	insulin treatment
8	S J	M	[illegible]	42	55	45	56	+	+	-	-	-	+	(+)	-	-	+	+	-	-	-	tolbutamide treatment
9	P G S	[illegible]	73	54	56	55	58	+	-	+	-	-	-	-	(+)	-	+	-	+	-	-	died from bleeding oesophageal varices Post mortem findings *liver cirrhosis*, *pancreatic fibrosis*

tolbutamide treatment	insulin treatment	pancreatic enzyme treatment tolbutamide treatment	relapsing duodenal ulcer pelvospondylitis	
-	-	-	-	
-	-	-	-	
+	-	+	-	—
-	+	-	+	
+	+	+	-	—
-	-	+	-	
+		+	-	
+	-	+	-	
-	-	+	-	
-	+	-		
	-	-	-	
+	-	-		
-	-	-		
+	-	-		
61	61	60	-	
55	50	63		
58	54	✓		
52	43	✓		
65	67	✓		
M	M	✓	✓	
G L	A B	✓		
10	11	✓		

			μg/l ...	GOT U	GPT U	Bromsulphthalein test retention after 25 min	after 45 min	Iv glucose tolerance test k-value
			1 2	39	20	3	2	0 36
			0 9	27	33	[illegible]		0 71
			0 9	19	40	6	1	0 71
			1 7	30	42	9	5	0 40
			1 1	11	9			1 15
			0 9	20	20	8	6	0 57
			0 9	15	17	11	3	0 51
			1 1	30	30	10	4	0 40
			[illegible] 5	60	20	32	24	0 55
			1 3	20	22	3	3	1 00
			0 9	26	32	12	4	0 43
			1 2	16	13		2	0 35
								0 91
		5	0 8	16	24	7	1	0 27
		6	1 0	25	23	9	3	1 90
		0 8	1 2	32	30			1 49
		0 8	1 [illegible]	14	12	9	4	[illegible] 96
		0 7	0 8	37	35			0 [illegible]
		0 8	1 3	34	20			1 19
	3	0 [illegible]	1 1	24	24	8	4	0 8
	0 6	0 9	0 9	3	35	8	4	0 67
	0 6	0 [illegible]	0 9	66	49	13	6	0 64
	0 4	[illegible] 8	0 8	[illegible]5	25	15	4	0 65
5	0 5	0 6	1 0	47	[illegible]3	[illegible]	5	0 53
0 3	0 8	0 7	1 2	[illegible]	46	6	3	1 15
0 3	0 5		1 6	13	9			1 67
				4	16			1 65

TABLE [illegible]

Subject				Age in years at		Specifications on pancreatitis						Remarks
						aetiology			symptoms and signs			
no	initials	sex	body weight kg	diagnosis of pancreatitis	present examination	alcoholism	prim biliary tract, disease	second biliary tract disease	diarrhoea	borborygmus	Heredity for diabetes	
28	A S	F	53	21	21	–	+	–	–	(+)		
[illegible]	G A S	F	84	24	27	–	+	–	+	+	–	op cholelithiasis
30	B h	F	112	25	27	–	–	+	–	+	–	
31	A G	F	53	36	39	–	+	–	–	+	–	op cholelithiasis
[illegible]	K S	F	55	41	44	–	+	–	+	+		
33	G C S	F	68	46	48	–	+	–	–	(+)	+	
34	I H	F	78	50	52	–	+	–	–	–	–	op cholelithiasis
[illegible]	H L.	F	74	51	60	–	+	–	–	–	–	
36	A K	F	63	59	62	–	+	–				op cholelithiasis
37	A S G	F	68	52	63	–	+	–	–	+	–	op cholelithiasis
38	E S	F	73	54	63	–	+	–	–	–	–	op cholelithiasis
39	E K	[illegible]	60	60	65	–	+	–	–	+	–	
40	S W	F	73	54	66	–	+	–	–	–	–	op cholelithiasis
41	L J	F	59	61	69	–	+	–	–	–	+	op cholelithiasis
42	B O	M	[illegible]	34	36	–	+	–	–	–	+	op cholelithiasis
43	J R	M	80	36	38	(+)	+	–	–	–	+	op cholelithiasis
44	A A	M	114	36	43	(+)	+	–	–	+	–	op cholelithiasis
45	Å L	M	83	46	48	–	+	–	–	+	+	op cholelithiasis
46	N H	M	87	44	50	–	+	–	–	–	–	op cholelithiasis
47	S S	M	74	50	52	–	+	+	–	–	–	op gastric ulcer op cholelithiasis
48	J A	M	87	[illegible]	61	–	+	–	–	–	–	op cholelithiasis
49	E H	M	70	60	65	(+)	+	–			–	op cholelithiasis
50	F S	M	60	67	71	(+)	–	–	–	+		

29

t

Initial	Amounts in the serum per 100 ml										Bromsulphthalein test retention		Iv glucose tolerance test
	bilirubin mg	alkaline phosphatase U	total protein g	albumin, g	$\alpha_1$globulin g	$\alpha_2$globulin g	βglobulin g	γglobulin g	GOT U	GPT U	after 20 min	after 45 min	k-value
R L	0 42	7 0	7 1	4 3	0 4	0 6	0 7	1 2	39	20	3	2	0 36
B J	0 27	6 9	6 9	4 1	0 5	0 7	0 8	0 9	27	33	2		0 71
D R	0 59	2 9	6 5	4 0	0 4	0 5	0 7	0 9	19	40	6	1	0 71
E O	0 51	3 9	7 8	3 7	0 2	0 9	1 3	1 7	30	42	9	5	0 40
G H	0 50	4 0	7 0	3 9	0 5	0 7	0 8	1 1	11	[illegible]			1 15
h A	[illegible] 40	5 0	6 6	4 0	0 4	0 6	0 7	0 9	20	20	[illegible]	6	0 57
G V O	0 56	4 2	6 5	4 4	0 3	0 5	0 7	0 9	15	17	11	3	0 51
S J	0 37	4 3	7 5	4 5	0 4	0 7	0 8	1 1	30	30	10	4	0 40
P G S	1 5	10	8 0	3 6	0 3	0 8	0 8	2 5	60	20	32	24	0 55
G L	0 59	2 9	6 9	4 1	0 3	0 5	0 7	1 3	20	22	3	3	1 [illegible]
A B	0 80	2 6	6 6	4 1	0 4	0 5	0 6	0 9	26	32	12	4	0 43
G N B	0 30	4	7 5	4 9	0 3	0 5	0 6	1 2	16	13		2	0 35
R S		6 0	7 0										0 91
T H			6 8	4 3	0 3	0 5	0 9	0 8	16	24	7	1	0 27
I L		2 4	6 6	4 3	0 3	0 4	0 6	1 0	25	[illegible]	9	3	1 90
M N	0 92	2 6	6 5	3 5	0 4	0 5	0 8	1 2	32	30			1 49
P O	0 95	5 8	7 4	4 5	0 3	0 6	0 8	1 2	14	[illegible]	9	4	0 96
B C	0 74	4 7	7 9	5 4	0 5	0 5	0 7	0 8	37	35			0 62
S W	0 30	4 0	6 7	3 6	0 3	0 7	0 8	1 3	34	[illegible]			1 19
E R B	0 29	2 2	7 4	5 1	0 2	0 3	0 7	1 1	24	24	8	4	0 8[illegible]
U T	0 32	8 3	6 8	4 0	0 4	0 6	0 9	0 9	37	35	[illegible]	4	0 67
B L	0 78	5 0	7 1	4 5	0 4	0 6	0 7	0 9	66	49	13	6	0 64
H G	0 75	2 6	6 7	4 4	0 3	0 4	0 8	0 8	25	25	16	4	0 65
B S	0 46	8 5	6 7	3 9	0 5	0 5	0 6	1 0	47	28	12	5	0 83
Å E	[illegible] 35	6 8	6 0	2 9	0 3	0 8	0 7	1 2	52	46	6	3	[illegible] 18
F N		5 0	7 4	4 4	0 3	0 5	0 7	1 6	13	9			1 67
E B	5	7 2							14	[illegible]			1 65

TABLE 29 (continued)

Subject no	Subject initial	bilirubin mg	alkaline phosphatase U	total protein g	albumin, g	$\alpha_1$globulin g	$\alpha_2$globulin g	βglobulin g	γglobulin g	GOT U	GPT U	Iv glucose tolerance test k-value
		Amounts in the serum per 100 ml										
28	A S	0 60	3 8	7 5	5 0	0 3	0 4	0 6	1 1	16	18	1 44
29	G A S	0 26	4 0	7 2	4 3	0 4	0 5	0 9	1 0	27	18	2 10
30	B K	1 06	4 2	7 5	4 4	0 3	0 7	0 9	1 2	30	36	0 94
31	A G	0 56	2 0	6 4	4 4	0 3	0 4	0 5	0 7	20	14	1 73
32	K S	0 70	4 0	6 6	4 4	0 3	0 4	0 5	1 0	26	21	1 49
33	G C S	0 70	7 0	6 3	3 3	0 3	0 6	0 9	1 2	18	28	1 82
34	I H	0 42	5 2	7 9	4 7	0 4	0 6	0 7	1 5	18	20	1 87
35	H L	0 37	4 0	7 1	4 5	0 3	0 4	0 8	1 1	18	15	0 74
36	A K	0 40	4 2	7 5	4 6	0 3	0 5	0 8	1 2	25	18	0 95
37	A S G	0 55	3 5	6 9	4 5	0 3	0 5	0 7	0 9	29	23	1 16
[illegible]	E S	0 61	14 0	7 4	4 7	0 3	0 5	0 7	1 2	33	22	1 24
39	E K	0 62	5 2	6 0	3 4	0 3	0 5	0 6	1 1	20	12	1 00
40	S M W	0 45	4 0	6 3	3 6	0 2	0 5	0 8	1 1	25	30	1 73
41	L S J	0 40	5 0	6 9	3 7	0 4	0 7	0 8	1 2	29	31	1 67
42	B O	1 1	6 5	7 6	4 8	0 3	0 5	0 7	1 3	27	28	1 42
43	J R	0 37	5 8	7 2	4 2	0 3	0 6	0 9	1 2	35	35	1 39
44	A T A	0 72	6 0	6 0	4 2	0 2	0 3	0 6	0 7	36	37	0 77
45	Å L	0 80	4 8	6 6	4 3	0 3	0 5	0 7	0 9	24	25	1 89
46	N H	0 48	6 0	7 0	4 8	0 2	0 5	0 7	0 7	28	37	1 33
47	S G S	0 72	8 8	6 9	4 1	0 4	0 5	0 8	1 1	26	25	1 15
48	J A	0 45	5 0	7 6	5 1	0 3	0 5	0 7	1 0	28	26	1 24
49	E H	0 92	2 1	7 2	4 4	0 3	0 5	0 8	1 2	25	47	0 94
50	F S	0 53	5 0	6 6	3 7	0 6	0 6	0 8	0 9	38	21	0 79

TABLE 30

Selection no	Variable no	C - Cp	C - Ap	Ap - Cp
2	1	*0 01708*	*0 13500*	*- 0 01276*
	2	- 0 68432	0 63450	- 0 06138
	3	[illegible]0 39072	-11 94810	16 71890
	4	0 09072	- 0 06000	0 03432
	[illegible]	[illegible] 25960	[illegible] 28200	4 00510
3	1	0 06524	0 09510	- 0 01694
	2	0 46284	- 0 28530	0 04202
	3	2 96352	- 4 03290	18 20302
	4	0 10024	- 0 08340	0 00946
	6	- 2 68128	- 1 15800	0 42570
	8	0 30884	0 26280	0 23188
	11	- 0 18004	- 0 37140	- 0 35442
4	1	0 09660	0 12870	0 00682
	2	- 0 81956	0 24840	- 1 18756
	3	20 63684	-10 79070	29 72728
	4	0 05124	- 0 10380	0 01540
	5	[illegible] 34600	- 0 58890	- 2 78652
	[illegible]	- 7 69804	- [illegible] 95550	- 1 01596
	7	10 94996	1 42620	13 55706
	8	0 30688	0 32070	0 71544
	9	- 0 32564	- 0 07020	- [illegible] 54868
	10	[illegible] 14924	- 0 01620	[illegible] 05346
	11	- 1 10460	- 0 46950	- 1 11408
5	1	0 02193	0 06000	0 04375
	5	3 90099	0 16750	3 85560
	12	- 1 59987	1 08250	0 19040
	13	52 49583	- 5 77600	14 59990
	14	[illegible] 34221	0 08550	0 23360
	15	- [illegible] 78511	- 3 65450	0 15855

TABLE 31 (continued)

Subject			Blood-glucose in mg/100 ml at following times in min									
no	initials	fasting value	10	[illegible]	40	60	80	100	120	140	160	180
44	A T A	88	323	335	339	349	238	133	80	63	60	56
45	Å L	81	279	298	288	271	109	53	44	47	50	53
46	N H	89	298	337	439	475	285	184	122	70	50	47
47	S G S	85	311	337	389	395	271	154	76	73	[illegible]	44
48	J A	84	347	372	459	485	291	198	108	72	53	46
49	E H	63	191	174	246	302	208	135	126	58	54	45
50	F S	74	288	323	359	383	330	263	212	158	119	94
52	M K	56	232	267	260	246	147	84	52	38	26	50
54	A J	62	285	263	256	256	152	83	76	58	51	55
61	I S	69	287	285	[illegible]	269	141	75	47	44	40	40
[illegible]	I B	68	242	252	265	255	112	62	52	[illegible]	[illegible]	65
63	R B	68	279	284	300	276	109	32		16	50	[illegible]
[illegible]	B M S	63	261	261	248	255	157	93	52	46	[illegible]	[illegible]
[illegible]	T R	91	263	278	324	300	179	103	[illegible]	80	[illegible]	80
66	A A	75	341	341	[illegible]	393	265	170	125	87	69	49
67	A N	83	266	270	319	315	211	155	100	70	[illegible]	[illegible]
68	K H	92	[illegible]	267	293	340	285	227	175	143	124	106
69	K L	85	344	360	445	535	303	222	144	104	63	54
70	E J	88	275	278	299	289	181	114	78	69	72	79
71	O A	99	302	325	365	385	268	205	142	105	85	78
73	R W	79	310	332	346	303	173	79	50	50	68	68
76	J T	65	239	300	364	230	147	109	60	41	41	63
79	K O	85	333	327	273	230	85	56	60	86	91	99
83	J M	62	245	245	278	258	172	75	52	42	55	58
[illegible]	K B	51	258	235	231	277	128	71	40	24	21	25
89	P M O	81	283	299	369	387	273	154	100	62	56	62
90	O E	79	290	260	245	246	130	54	61	63	63	71
92	L J	67	330	338	418	433	271	163	71	57	33	29
93	T W	75	282	277	313	324	149	58	35	33	40	46
94	S L	80	302	338	423	457	245	137	74	41	35	41
96	C S	99	297	335	305	358	255	153	107	74	54	51
97	E L	80	252	281	315	312	215	115	65	[illegible]	52	59
98	G D	81	218	231	251	268	195	155	112	98	[illegible]	88
99	J S	99	298	333	407	433	298	195	150	107	85	[illegible]
100	H W	92	298	362	[illegible]	349	227	137	[illegible]	69	[illegible]	49

TABLE 31 (continued)

Glycosuria g/3 hours	fasting value	10	20	40	60	80	100	120	140	160	180
	Plasma-insulin in μU/ml at following times in min										
19 1	62	235	240	265	280	360	290	177	110	84	82
14 5	41	54	80	74	130	143	70	34	38	52	49
15 2	58	89	115	98	214	220	210	190	66	64	62
22 4	45	71	81	105	180	167	190	165	76	62	[illegible]
8 1	62	230	210	205	340	360	320	225	135	58	78
10 8	42	46	41	47	66	74	74	71	53	46	44
4 0	31	40	44	63	59	59	60	64	53	52	47
6 1	56	57	98	73	79	115	90	61	65	56	44
6 7	43	12[illegible]	147	14[illegible]	160	110	56	46	44	41	34
11 8	46	112	116	115	116	130	76	72	61	53	56
6 0	52	74	76	94	125	89	59	54	56	54	54
7 0	50	112	132	140	180	149	76	71	49	51	51
3 8	41	135	140	110	97	86	48	40	38	38	[illegible]
6 7	56	68	70	76	85	80	58	50	53	47	47
12 7	53	117	82	11[illegible]	210	140	135	110	74	76	51
[illegible] 4	44	86	83	115	140	190	107	74	54	[illegible]5	[illegible]
7 8	49	51	46	52	66	61	54	75	56	45	44
[illegible] 4	60	160	148	180	240	255	235	212	145	34	76
10 5	43	66	93	128	156	100	69	46	44	43	45
7 2	76	100	107	112	130	125	108	80	60	52	[illegible]
14 [illegible]	56	69	66	95	180	190	91	68	65	54	51
9 0	49	54	71	73	70	91	58	57	54	51	52
5 6	60	145	176	205	245	107	65	62	55	59	
6 2	47	[illegible]	63	84	148	128	70	58	38	34	45
10 7	34	39	44	44	56	48	56	38	34	33	42
16 5	47	55	54	70	84	100	92	80	49	51	42
3 3	45	82	[illegible]	65	78	72	53	46	42	[illegible]1	47
1 2	16	84	84	110	167	185	14[illegible]	125	72	[illegible]	45
16 3	15	70	69	86	134	148	62	52	53	56	[illegible]
16 8	53	76	76	114	156	143	153	83	60	4[illegible]	[illegible]2
13 0	54	115	150	190	330	350	335	195	12[illegible]	[illegible]	72
8 5	49	57	[illegible]0	48	68	72	58	51	[illegible]6	44	47
5 1	47	48	[illegible]4	64	60	69	49	47	44	42	[illegible]
12 6	[illegible]8	49	[illegible]0	58	82	88	[illegible]0	74	57	[illegible]	47
11 7	77	96	79	94	125	125	165	93	110	1[illegible]7	116

TABLE 32

Subject		Glucose space		$\log k_{i1}$	$\log k_{i2}$	log b	$\log k_g$	Glucose uptake in g and in per cent of given amount			
no	initials	liter	per cent of body wt					0 – 60 min		0 – 180 min	
								g	%	g	%
3	D R	[illegible] 0	31 9	1 784	2 879	1 863	1 363	29 7	24 4	101 2	83 3
5	G H	16 5	25 1	<1 000	2 827	1 966	1 534	37 8	35 4	103 3	96 6
8	S J	15 8	26 8	<1 000	1 748	<1 000	1 155	9 3	9 8	37 7	39 4
9	P G S	21 1	28 9	1 117	2 586	1 872	1 043	16 3	15 5	64 2	71 2
10	G L	18 [illegible]	26 6	<1 000	1 924	1 332	1 333	18 7	[illegible] 5	74 3	65 6
12	G N B	16 7	32 1	<1 000	<1 000	<1 000	1 110	5 3	6 3	19 0	22 6
13	R S	18 9	25 8	2 105	2 164	1 447	1 209	26 5	22 4	89 5	75 7
15	I L	18 1	30 2	2 117	2 301	1 964	1 664	39 0	40 1	88 [illegible]	90 [illegible]
16	M N	15 1	21 5	1 955	2 261	1 740	1 391	28 7	25 3	88 5	78 0
17	P O	16 8	27 1	1 755	2 614	1 829	1 636	35 7	35 5	93 7	93 3
18	B C	20 1	29 2	2 023	2 602	2 254	1 526	50 5	45 2	101 3	90 7
19	S W	14 7	21 5	1 308	2 853	2 061	1 332	25 0	22 7	93 0	84 4
20	E R B	20 2	31 0	<1 000	2 564	1 826	1 307	24 2	23 0	91 3	86 9
21	U T	16 8	27 9	<1 000	2 395	1 820	0 840	7 5	7 7	40 6	41 7
22	B L	19 2	33 1	1 695	2 6[illegible]0	1 695	1 338	21 8	23 2	75 5	60 4
23	H G	17 5	21 8	1 680	2 354	1 803	1 418	31 0	23 9	106 0	81 8
24	B S	23 2	22 0	2 011	2 636	2 166	1 096	20 2	15 6	100 3	77 4
25	Å E	17 5	30 6	<1 000	1 558	<1 000	1 623	28 0	30 3	81 8	88 6
26	F N	21 7	32 8	2 560	3 036	2 461	1 282	44 2	41 3	108 0	101 0
27	E B	20 4	31 9	1 751		1 752		37 7	36 3		
28	A S	17 2	32 4	2 049	2 990	2 469	1 445	35 2	41 0	85 5	99 6
29	G A S	25 3	30 3	2 645	3 086	2 482	1 190	44 5	32 7	122 8	[illegible] 3
30	B K	24 4	21 6	1 731	3 168	2 277	1 263	46 7	25 7	163 7	90 2
31	A O	13 2	24 9	<1 000	2 254	1 927	1 567	25 8	30 1	65 8	76 7
32	[illegible] S	14 7	26 6	1 612	2 1[illegible]5	1 875	1 758	35 5	39 8	79 5	89 2
33	G C S	15 6	23 0	1 872	2 808	[illegible] 646	1 190	37 7	34 2	100 7	91 4
34	I H	18 3	23 5	2 212	[illegible] 466	2 259	1 492	50 8	40 2	119 2	94 3
35	H L	16 0	24 4	<1 000	3 117	1 954	1 369	22 3	18 6	106 0	90 5
36	A K	14 [illegible]	23 1	1 281	2 211	1 690	1 240	24 8	24 3	78 0	76 4
37	A S G	15 3	22 5	1 762	2 015	2 417	1 142	22 8	20 7	90 5	82 2
38	E S	17 3	[illegible] 7	1 820	2 788	2 017	1 506	37 7	31 9	109 5	92 6
39	E K	17 6	29 3	1 453	2 356	2 070	1 500	30 [illegible]	31 4	87 8	90 4
40	S M W	14 2	19 4	1 894	2 642	2 462	1 233	32 2	27 2	104 2	[illegible] 1
41	L S J	12 7	21 5	2 696	3 152	2 500	1 135	33 0	34 5	92 7	97 0
42	B O	19 6	28 8	2 151	2 699	2 309	1 313	31 0	28 1	99 7	90 5

TABLE 32 (continued)

Subject		Glucose space		log k_{11}	log k_{12}	log b	log k_g	Glucose uptake in g and in per cent of given amount			
no	initials	liter	per cent of body wt					0 – 60 min		0 – 180 min	
								g	%	g	%
43	J R	19 6	24 6	2 300	2 714	2 223	1 500	57 5	44 4	136 7	106 0
44	A T A	25 8	22 6	2 889	3 374	2 689	1 055	79 3	43 0	170 0	92 1
45	Å L.	24 1	29 0	1 871	2 899	2 265	1 528	51 0	37 9	123 8	92 1
46	N H	19 4	22 3	2 263	3 013	2 462	1 157	42 7	30 3	126 0	89 4
47	S G S	18 4	24 8	2 049	3 218	2 308	1 238	30 3	25 3	106 7	89 0
48	J A	17 6	20 2	2 783	3 278	2 699	1 001	53 3	37 8	136 2	96 8
49	E H	18 3	24 4	2 050	2 821	2 290	1 302	36 2	27 3	106 2	87 4
[illegible]	F S	16 [illegible]	27 0	1 769	2 242	1 591	1 035	22 0	22 6	78 7	80 9
52	M K	15 7	29 1	2 418	2 8 5	2 066	1 609	41 7	[illegible]	87 2	99 6
54	A J	15 8	28 7	2 651	2 702	2 511	1 345	42 3	47 5	83 8	94 1
61	I S	15 6	26 5	2 505	2 553	2 337	1 460	45 5	47 6	86 0	90 0
62	I B	19 7	31 7	2 100	2 592	2 204	1 565	50 2	49 9	100 5	100 1
63	R B	17 0	[illegible] 6	2 413	2 902	2 481	1 384	52 3	48 1	109 3	100 5
[illegible]	B M S	19 0	28 8	2 723	2 517	2 158	1 470	54 3	50 8	102 3	95 7
[illegible]	T R	18 0	31 5	1 822	2 321	1 806	1 597	37 8	41 0	87 2	94 4
66	A A	1 [illegible]	21 8	2 300	2 648	2 502	1 259	39 8	35 6	100 7	90 1
67	A N	19 7	31 8	2 333	2 865	2 537	1 242	34 3	34 2	97 8	97 4
68	K H	15 9	33 1	$<$1 000	2 232	1 146	1 551	21 7	27 9	66 8	85 9
69	A L.	12 7	20 8	2 543	2 945	2 511	1 072	33 7	32 5	99 5	95 9
70	E J	20 0	31 7	2 049	2 680	2 418	1 400	43 0	40 1	99 3	92 7
71	O A	14 2	[illegible]	2 1[illegible]6	2 338	2 111	1 287	28 5	33 2	7[illegible] 2	87 5
73	R W	19 7	26 9	1 886	2 929	2 424	1 342	43 3	36 6	114 [illegible]	96 5
76	J T	22 5	32 1	2 246	2 873	2 258	1 714	72 2	60 7	117 5	98 7
79	K Ö	14 9	18 6	2 547	3 006	2 638	1 524	101 3	74 5	133 8	98 4
83	J M	20 1	32 5	2 166	2 787	2 261	1 516	44 2	44 0	101 5	101 1
88	K B	16 6	2[illegible] 9	1 255	3 0[illegible]0	1 491	1 927	54 5	[illegible] 1	105 5	97 0
[illegible]	P M O	[illegible] 8	26 6	1 562	2 670	1 698	1 479	36 0	28 5	111 3	88 1
90	O E	18 2	27 2	2 127	2 369	1 724	1 857	73 7	64 7	113 3	99 5
92	L J	21 4	23 2	2 164	2 846	2 344	1 324	[illegible] 3	34 7	160 0	102 3
93	T W	[illegible] 0	26 8	2 076	2 898	2 260	1 509	60 3	43 3	132 0	94 7
94	S L.	17 0	[illegible] 4	1 892	2 870	2 290	1 422	42 0	32 5	118 5	91 7
96	C S	22 5	29 2	2 530	3 242	2 654	0 982	38 7	29 6	124 3	95 0
97	E L	21 0	29 2	1 477	2 253	1 644	1 719	53 8	44 0	116 0	94 8
98	G D	25 3	35 6	$<$1 000	2 214	1 857	1 611	41 2	35 8	106 7	92 7
99	J S	16 2	26 6	1 415	2 400	1 914	1 384	31 2	30 1	91 5	88 2
100	H W	19 4	26 6	1 839	2 737	2 017	1 427	40 0	32 2	115 7	93 2

TABLE 33

Subject		Volume ml – Bicarbonate mE in period number							Max bicarbonate concn mE/l	Amylase AU – Trypsin mg in period number						
no	initials	I	II	III	IV	V	VI	VII		I	II	III	IV	V	VI	VII
1	R L	27 0 35	55 1 60	37 0 89	13 0 31	12 0 31			29 0	3[illegible] 1 7 08	9 7 8 44	4 4 0 59	1 2 0 31	5 5 0 73		
2	B J	66 1 34	84 3 27	51 2 01	30 1 15	27 1 57	61 1 15	39 1 02	58 8	28 8 9 92	19 8 4 47	9 0 1 48	9 3 1 79	13 6 2 32	44 [illegible] 6 40	28 2 12 00
4	E O	27 0 12	66 2 03	58 1 67	36 1 15				32 1	11 1 14 41	21 7 17 64	16 1 6 08	14 2 1 94			
5	G H	38	52	40	31	44 8 89 (←II–V)								28 69 (←II–V)		
6	K Å		8 0 09	8 0 10	25 0 08	25 0 00			12 5		19 4	13 4	5 9	0 2		
7	G V O	41 0 62	51 2 59	[illegible] 0 84	26 0 84	13 0 48			50 4	6 2 1 23	8 6 1 51	5 7 1 47	6 2 1 00	3 5 1 58		
8	S J	45 3 78	71 2 34	51 2 76	59 2 93	57 1 74	50 2 50	92 3 86	84 0	42 8 6 58	102 9 19 27	40 3 8 90	43 9 15 62	49 0 11 16	60 4 18 72	69 5 28 18
9	P G S	116 4 36	1[illegible]6 7 49	158 7 58	174 9 26	96 3 70	79 2 56	52 1 98	53 2	56 5 10 6[illegible]	23 4 4 [illegible]	12 6 2 [illegible]3	92 6 0 71	86 6 2 82	26 4 7 42	17 2 3 95
10	C L	55 0 [illegible]	52 1 0[illegible]	40 0 84	33 0 77	20 0 45	25 0 53	20 0 40	23 1	0 1 1 37	1 6 5 25	0 4 2 27	0 9 5 08	0 7 5 40	1 0 7 74	0 8 6 00
11	A B	21 0 46	34 1 18	30 1 13	13 0 58	10 0 60			60 4	14 3 2 12	24 1 2 66	12 9 1 39	6 2 0 93	4 3 1 15		

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12	G N B		46 0 19	26 0 06	20 0 24	8 0 10			14 7		7 8	4 7	2 6	14 9		
13	R S		61 4 73	50 4 55	46 4 48	49 4 [illegible]			107 1					369 6		
[illegible]	T H	14 0 32	30 0 92	11 0 28	14 0 65		21 0 91	[illegible] 0 60	45 4	5 0 0	47 3 2 99	5 9 1 06	8 8 2 02		33 8 7 20	4 8 12 94
16	M N	41 2 31	102 8 24	82 8 27	37 3 76	28 2 52			100 5	141 1 22 18	206 0 62 49	165 3 [illegible] 81	95 2 8 68	41 7 4 11		
17	P O	29 1 62	[illegible] 0 [illegible]	10 0 51	7 0 25	16 0 38			56 6	54 8 5 68	38 7 4 86	12 4 1 29	3 2 0	14 4 0		
18	B C	68 3 60	79 4 00	30 0 71	73 4 08		50 2 92		62 2	219 1 30 04	111 2 30 00	71 0 18 71	214 1 23 33		201 4 36 72	
19	S H	39 1 49	73 3 12	74 4 16	65 1 50	75 0 82	65 0 80	52 0 71	50 2	84 9 24 80	81 2 20 53	71 8 33 95	164 9 10 78	197 7 10 30	164 0 44 76	49 2 [illegible] 78
20	E R B	[illegible] 0 76	71 2 13	64 2 42	57 2 51		45 2 24	47 2 33	48 0	130 4 9 19	90 9 18 63	175 6 10 13	220 2 7 02		205 9 6 31	175 6 6 10
21	U T	[illegible] 0 85	86 1 [illegible]	65 2 18	39 1 21		[illegible] 1 26		33 7	130 5 33 55	62 8 21 94	88 4 11 [illegible]	50 7 7 22		121 0 17 39	
22	B L	58 2 40	96 5 76	[illegible] [illegible]	63 4 91	49 4 02			82 0	103 2 42 42	106 6 43 [illegible]	72 2 38 57	69 9 30 87	37 2 15 78		
23	H G	45 2 07	35 2 14	4 0 35	65 3 71	36 1 67	77 7 18	59 5 67	88 5	274 5 16 07	169 8 3 57	12 2 0 28	212 8 5 86	71 9 5 85	312 0 31 94	176 3 33 12
24	D S	51 1 56	54 2 08	81 3 88	66 3 95	70 3 61	90 3 71	75 1 37	[illegible] 5	263 3 14 82	153 6 8 00	350 0 7 56	97 4 16 47	259 8 26 30	383 8 35 21	168 0 17 60
25	Å E	55 0 84	72 1 73	14 0 14			117 2 14		21 1	53 4 10 44	29 3 8 40	2 0 0			18 9 12 35	
26	F N		58 3 32	64 4 14	47 3 13	30 1 97			85 1		1321 1					

TABLE 33 (continued

Subject		Volume ml – Bicarbonate mE in period number							Max bicarbonate concn mE/l	Amylase AU – Trypsin, mg in period number						
no	initials	I	II	III	IV	V	VI	VII		I	II	III	IV	V	VI	VII
5[illegible]	O B	 4 88	94 7 43	[illegible] 7 2[illegible]	42 3 79	4[illegible] [illegible] 86			129 2	419 4	3[illegible]8 7	215 6	[illegible]	54 0		
56	G S	 4 98	84 8 97	75 8 19	60 7 24	70 8 15			120 1	178 7	487 8	141 6	159 7	174 8		
57	V L	 2 92	83 6 41	107 6 34	76 4 81	55 5 5[illegible]			100 7	157 0	186 3	302 4	100 9	68 3		
[illegible]	G O	 2 3[illegible]	47 4 02	57 1 2[illegible]	66 4 90	47 5 62			120 7	332 9	172 7	18 2	123 5	101 2		
59	M G	 3 [illegible]	75 7 [illegible]	63 6 34	61 6 08	53 5 66			107 4	139 9	164 4	89 1	71 3	47 0		
60	C H	 [illegible] 30	96 8 [illegible]	71 7 48	84 8 60	79 6 12			104 8	177 6	206 3	97 0	112 1	19[illegible] 7		
61	I S	 4 11	70 5 [illegible]	57 5 79	[illegible] 4 [illegible]	57 [illegible] 53			101 9	371 8	321 5	198 6	191 9	144 3		
62	I B	9[illegible] 4 73	137 11 13	100 8 77	83 7 57	123 10 03	151 11 70	84 7 [illegible]	[illegible] 6	227 4 28 91	232 3 30 09	193 8 15 50	156 5 17 54	248 [illegible] 44 88	4[illegible]5 8 122 [illegible]	130 7 42 31
63	R B	80 4 21	178 8 54	98 5 [illegible]	62 5 70	[illegible] 5 [illegible]	94 5 64	56 3 [illegible]	96 7	331 3 3[illegible] 64	336 4 44 14	201 9 19 60	166 8 7 44	10[illegible] 8 7 39	340 [illegible] [illegible] 36	136 0 22 03
64	B M S	61 2 14	104 4 78	55 3 30	50 4 50	40 3 12			[illegible] 1	233 7 21 17	242 [illegible] 21 10	[illegible] 7 4 40	116 5 4 40	81 2 5 [illegible]		

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
65	T R	91 6 08	124 10 [illegible]	92 7 64	89 9 20	[illegible] 8 90	110 9 16	84 8 [illegible]	106 5	26 6 37 24	45 4 52 92	19 6 23 91	22 1 17 30	20 2 12 43	44 8 94 08	8 4 32 93
66	A A	40 2 04	57 7 32	73 6 24	69 5 48		121 6 82		128 4	514 7 5 13	260 5 4 70	283 1 2 73	218 3 3 [illegible]8		409 2 13 02	
67	A N	42 2 88	52 4 97	47 4 00	49 4 58	49 4 20			96 3	300 6 21 78	211 4 11 23	176 5 6 00	212 3 6 86	258 0 6 00		
68	K H	59 3 47	78 [illegible] 12	[illegible] 5 18	87 6 12	7 0 34			75 0	26[illegible] 4 26 04	214 4 32 00	157 0 11 52	275 4 28 08	10 2 1 51		
69	K L	75 3 43	78 7 4[illegible]	81 7 22	66 6 26	[illegible] 4 70			9[illegible] 7	546 7 66 00	315 9 23 27	228 0 10 82	201 3 10 88	193 8 8 51		
70	E J	61 3 78	88 8 54	70 7 00	67 7 18	69 7 10			107 7	240 0 21 92	161 1 17 41	114 1 9 21	179 4 14 3[illegible]	128 5 7 93		
71	O A	3[illegible] 1 2[illegible]	74 4 20	39 1 80	70 2 90	66 3 08			57 1	118 6 8 74	13[illegible] 8 9 90	4[illegible] 9 3 77	137 6 6 16	136 5 17 08		
72	H O I	2[illegible] 0 43	75 4 37	59 4 03	70 4 44		81 3 27	73 4 951	68 2	76 0 8 0[illegible]	225 4 9 0[illegible]	61 7 5 29	202 0 0		404 4 125 [illegible]	[illegible] 5 35 70
73	R W	 5 84	111 9 42	118 8 89	7[illegible] 3 45	38 1 58			65 0	206 6	312 7	276 5	131 9	84 4		
74	D E	 5 88	157 12 78	129 13 02	126 11 68	9[illegible] 10 19			107 2	134 8	101 7	42 2	76 6	92 0		
75	J J	 3 56	119 11 00	99 8 31	45 3 69	47 4 07			92 2	236 6	278 7	237 1	117 6	9[illegible] 0		
*76	J T	62 3 90	97 9 90	8[illegible] 8 77	110 11 6[illegible]	49 4 70	168 10 9[illegible]	106 8 42	10[illegible] 8	328 9 17 91	330 0 2[illegible] 34	287 2 23 74	415 5 30 24	19[illegible] 0 14 00	734 1 151 55	360 7 54 43
77	S S	 4 66	122 8 93	[illegible] 8 64	104 9 26	98 7 30			9[illegible] 9	497 5	432 2	233 1	211 5	173 7		
78	S R		152	101	8	100										

TABLE 33 (continued)

Subject		Volume ml — Bicarbonate mE in period number							Max bicarbonate concn mE/l	Amylase AU — Trypsin mg in period number						
no	Initials	I	II	III	IV	V	VI	VII		I	II	III	IV	V	VI	VII
79	K Ö	101 4 94	165 8 74	[illegible] 7 60	84 8 70	74 6 69	118 10 [illegible]	64 5 [illegible]	103 3	277 1 54 80	332 6 34 94	198 0 10 00	186 2 12 18	168 7 10 42	50[illegible] 0 108 56	139 5 47 87
[illegible]	J T A	 3 [illegible]	90 7 31	48 2 85	85 5 74	75 6 26			113 4	283 5	385 3	123 6	245 2	233 4		
[illegible]	H E	41 2 25	97 7 70	105 7 02	107 7 51		130 7 02	[illegible] 6 63	90 6	56 5 2 21	184 6 5 71	106 5 5 10	121 9 5 10		309 2 14 18	133 5 13 60
[illegible]	S B	120 0 02	119 4 23	64 2 59	81 3 62	71 3 32	144 6 69	111 6 09	[illegible] 6	 3 86	177 4 5 66	77 0 2 50	108 7 2 99	83 6 2 44	322 9 12 20	204 9 12 71
83	J M	93 5 32	118 6 78	128 6 13	58 4 52	73 5 53	98 7 47	[illegible] 5 54	89 0	202 3 20 90	154 9 26 62	162 3 23 30	130 0 16 67	126 0 18 29	451 5 78 78	195 7 47 04
84	S [illegible]	68 3 59	105 8 60	75 4 14	124 5 58		168 11 44	100 8 11	97 2	236 1 8 [illegible]	147 8 9 96	32 4 1 60	83 4 2 10		420 8 48 80	125 5 25 00
85	R E	61 4 10	102 8 74	75 7 43			114 9 69		99 0	228 7 12 35	283 9 8 01	129 0 9 45			430 9 101 35	
86	H R	76 5 37	109 10 75	80 7 82	72 7 08	73 8 11	108 6 54	84 7 57	111 5	81 4 38 08	163 5 [illegible] 70	130 9 20 06	105 5 20 33	141 2 28 70	394 6 85 [illegible]	117 8 29 24
87	P S	26 1 22	79 3 32	47 4 32	[illegible] 4 00		100 5 30		93 0	47 1	131 9	87 4	24 9		142 0	
88	K B	48 2 25	72 6 16	52 4 70			[illegible] 6 87		90 4	178 5 9 50	144 0 10 78	48 1 2 70			233 2 21 [illegible]	

89	P M O	72 3 80	124 11 32	91 9 28	106 8 62	103 6 [illegible]			96 9	72 [illegible] 28 31	153 5 62 70	51 9 24 [illegible]	[illegible] 0 25 15	50 0 20 74		
90	O E	23 1 43	76 6 [illegible]	35 2 16			97 6 [illegible]		80 5	158 9 20 93	568 0 38 71	158 0 9 62			712 1 138 57	
91	P T	30 2 95	74 6 62	50 6 01			86 7 92		102 2	198 8 7 84	387 2 11 40	115 3 5 21			415 8 50 16	
92	L J	20 0 43	107 4 70	133 6 21			87 1 06	101 4 91	46 6	58 9 9 98	127 7 15 [illegible]	141 9 13 52			155 6 8 51	113 1 17 96
93	T W	110 7 10	148 10 36	113 9 35	95 9 70	89 7 48	180 10 08	106 9 02	100 9	639 4 31 52	424 6 41 40	196 1 25 08	190 1 8 83	191 4 18 51	780 0 180 00	350 4 52 80
94	S L	103 7 [illegible]	136 9 11	101 8 36	91 0 02	85 8 10	154 9 30	104 7 21	98 9	673 5 37 21	781 1 6[illegible] 93	356 4 35 64	206 4 14 21	252 6 12 67	677 4 166 78	383 7 78 86
95	S R	110 7 50	116 7 55	168 12 01	80 6 40	155 12 70	277 18 68	124 9 07	81 8	592 9 41 48	338 7 19 82	433 6 13 07	205 8 6 20	337 8 35 42	915 2 176 50	101 0 47 [illegible]
96	C S	107 4 84	131 8 04	118 7 68	119 8 30	113 6 50			65 6	356 4 80 76	318 4 65 22	258 0 63 81	230 0 56 61	205 3 48 26		
97	L L	76 4 80	93 8 [illegible]	58 4 72	71 6 62	128 7 50			93 1	372 8 23 68	287 0 22 40	158 7 7 08	154 8 8 64	242 6 21 44		
98	G D	30 1 37	45 3 38	90 6 32	59 5 61	57 3 60			95 3	85 1 12 01	61 1 7 05	133 9 12 28	108 0 9 52	83 4 0 00		
99	J S	33 1 82	63 5 01	40 3 86	43 1 05	55 4 05			91 6	191 6 12 60	192 8 16 53	136 2 7 73	169 3 6 51	191 2 8 73		
100	H W	79 6 56	89 8 01	80 6 80	76 7 70	73 7 50			101 0	205 7 29 52	172 9 42 95	138 5 35 32	187 1 21 64	117 8 9 [illegible]		

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ANASTOMOSES BETWEEN EXTRACARDIAC VESSELS AND CORONARY ARTERIES

by

ANDERS MOBERG

STOCKHOLM 1968

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CONTENTS

Introduction

The leading cause of death in western countries is an inadequate myocardial blood supply (World Health Statistics Annual 1964) The coronary arteries were once considered to be end arteries but Spalteholz (1924) and Schlesinger (1938) demonstrated a network of anastomosing vessels between these arteries Later investigators confirmed the existence of intercoronary anastomoses and also showed that in hearts with stenosis of only one of the coronary arteries, the anastomoses can enlarge and to some or a major extent take over the blood supply of the part of the myocardium formerly supplied by the stenosed artery (Blumgart et coll 1940, Laurie & Woods 1958, Fulton 1965)

In ischemic heart disease intercoronary anastomoses can only redistribute the blood supplying the myocardium but cannot increase the total blood supply to the myocardium (Bloor & Liebow 1965) An increase in the total blood supply to the myocardium must come either from within the heart—endomural anstomoses—or from extracardial sources Almost nothing is known about collateral function of the endomural anastomoses (Bloor & Liebow 1965) On the other hand, anastomoses between extracardial vessels and the coronary arteries have been known since the beginning of the 19th century (v Haller 1803) But the natural occurrence function and possible importance of extracardial anastomoses as a source of blood to the ageing myocardium have attracted surprisingly little attention

The pathways for a blood supply from extracardial vessels to the myocardium could be through the pericardium but this requires the presence of pericardial adhesions In the majority of human beings without such adhesions a blood supply from extracardial sources must pass through the pericardial reflexion The parietal pericardium receives its blood supply via branches from the bronchial arteries and the internal mammary arteries, (arteria thoracica interna according to current anatomical nomenclature) These two arterial systems run close to the pericardial reflexion From this region the possible pathways for an extracardial blood supply could be either via the vasa vasorum of the aorta or via the atrial arteries vessels which arise from the main coronary arteries

The present series of studies were performed in order to find out

(i) the extent of anastomoses between the bronchial and the coronary arteries

(ii) the extent of anastomoses between the internal mammary and the coronary arteries

(iii) the microangiographical appearance of the extracardial anastomoses,

(iv) the possibilities of demonstrating these anastomoses in living subjects

The results of this series of studies have been reported in the articles listed below, an initial report was presented at the meeting of the Swedish Pathological Society, November 30, 1963 These articles constitute the basis for the following survey and discussion They will be referred to by Roman numerals

I Håkan Arvidsson and Anders Moberg Extracardiac anastomoses to the myocardium Preliminary report of angiocardiographic and anatomic studies Acta radiol Diagnosis 4 385—394, 1966

II Anders Moberg Anastomoses between extracardiac vessels and coronary arteries — I — via bronchial arteries Postmortem angiographic study in adults and newborn infants Acta radiol Diagnosis 6 177—192, 1967

III Anders Moberg Anastomoses between extracardiac vessels and coronary arteries — II — via internal mammary arteries Post-mortem angiographic study Acta radiol Diagnosis 6 263—272, 1967

IV Anders Moberg Anastomoses between extracardiac vessels and coronary arteries — III — microangiographic appearance Acta radiol Diagnosis 7 33—47, 1968

Review of Literature on Extracardial Coronary Anastomoses

Anatomical studies

The first description of extracardial anastomoses dates back to v Haller 1803 He found communications between the coronary arteries and the mediastinal vessels The first more comprehensive study was made by Hudson, Moritz & Wearn 1932 Working with human post-mortem material they injected the coronary arteries with India ink with a pressure of 220 mm Hg Detailed information is not given but it is stated that anastomoses were found between the coronary arteries and the pericardiophrenic branches of the internal mammary arteries, the anterior mediastinal pericardial, bronchial superior and inferior phrenic, intercostal and oesophageal branches of the aorta The number of anastomoses increased with age but the increase was not considered in relation to the degree of coronary atheromatosis In conclusion the authors stated that "this rich potential extracardiac coronary collateral circulation is probably of significance in compensating for sclerosis of the large trunks of the coronary arteries

Recently Petelenz (1963 1965 a, b c) studied anastomoses between the bronchial and the coronary arteries in 100 adult subjects The results of post-mortem contrast injection into the bronchial arteries were that in 37 specimens contrast medium "reached the heart but did not spread in it and in 40 specimens contrast medium "covered (to different degrees) the auricular surface The right coronary artery was filled in 10 hearts and the left in 4 more hearts. In 26 specimens contrast medium was visible in the region of the sinus node. Extracardial anastomoses could be identified to a greater extent in patients suffering from "arteriosclerosis with coronary disease , but it is not stated whether the degree of coronary stenosis was measured. The articles lack information about the amount of contrast medium injected and the radiological information is scanty

Apart from these two reports, anastomoses between the bronchial and the coronary arteries in human beings have been mentioned only briefly—Koch (1909) Spalteholz (1924), Schoenmackers & Vieten (1954), Fulton (1965) The anastomoses seem to have been found accidentally during investigation of other but related problems.

Ligation of the internal mammary arteries was devised as a new operation for ischemic heart disease by Fieschi in 1942 The idea behind the operation was to direct blood-flow to the pericardiophrenic arteries Originating from the first part of the internal mammary arteries these vessels give off branches to the pericardium.

This operation has been abandoned in most clinics but in its time it stimulated an increased interest in anastomoses between the internal mammary arteries

and the coronary arteries Most of the work was performed on dogs but, unlike human beings, dogs have large anastomoses between the bronchial and the internal mammary arteries (Berry et coll 1931, Horine & Warner 1932, Cauldwell et coll 1948) and conclusions about the source of an extracardial blood supply cannot be drawn from experimental work on that animal

Few reports deal with the anatomical demonstration of anastomoses between the internal mammary and the coronary arteries in human beings Fieschi (1942) ligated the internal mammary arteries in the second intercostal space and at the origin from the subclavian artery Injection of the isolated part of the internal mammary artery filled the vasa vasorum of the aorta and pulmonary artery Battezzatti et coll (1955) repeated the experiments and demonstrated filling of vessels in the myocardium and epicardial fat Unfortunately, these two articles lack information about the number of subjects injected, age degree of coronary atheromatosis as well as the presence or absence of pericardial adhesions

Vascularisation via pericardial adhesions has been described after disease (Moritz Hudson & Orgain 1932) and after surgical procedures (Plachta, Thompson & Speer 1955) The present investigation concerns the natural extent of extracardial anastomoses and since transpericardial vascularisation requires a previous pericardial disease, it will not be dealt with in the following

Clinical studies

The first angiographic demonstration of extracardial anastomoses in human subjects was published by Di Guglielmo in 1960 During a conventional coronary angiography, wide and tortuous mediastinal vessels, with the appearance of bronchial arteries, were seen to fill the circumflex branch of the left coronary artery distal to an occlusion

After the first article in the present series was published, Bjork (1966 b) reviewed 200 coronary angiographies on living subjects An anastomotic flow from the bronchial to the coronary arteries was identified in 73 The anastomoses were visible more often in subjects with coronary atheromatosis 53 of 109 (49 per cent), than in subjects with angiographically normal coronary arteries, 20 of 91 (22 per cent) In 14 subjects with coronary atheromatosis the anastomoses had a diameter of at least 2 mm as measured from the angiograms

Bjork (1966 a) has also demonstrated an anastomotic flow between the bronchial and coronary arteries in subjects with Fallot s anomaly or pulmonary atresia In these subjects, however, the flow is reported to have been from the coronary to the bronchial arteries A "bronchial steal syndrome' was demonstrated in 12 of the 67 patients

Frequency and Morphology of Extracardial Coronary Anastomoses

MATERIAL AND METHODS

Autopsy series

The studies (I—IV) covered 177 subjects but 10 hearts had to be discarded from the series (II and III), the first 35 were included in the initial report (I) and the basic material for discussion here is then 132 subjects It consists of 8 new-born infants, 20 specimens from subjects between 10 and 50 years of age, and 29, 28, 40 and 7 subjects from the 6th 7th, 8th and 9th decade respectively There is a male predominance in the adult material, 72 against 52 females

The material was obtained at consecutive personally performed autopsies and the general data given below apply to the three series (II III and IV) There were no major differences in the composition of the series The specimens were taken from subjects for which the major autopsy diagnosis were non-vascular extrathoracic diseases (61) vascular disease of the brain (21) myocardial infarction (16), generalised atheromatosis with heart failure (11), pulmonary embolism (6), renal diseases with renal failure (3), ruptured aortic aneurysm (2), valvular heart disease (1) and pulmonary tuberculosis (1) In addition to the 6 patients which died from pulmonary embolism, 12 more patients had small pulmonary emboli Pericardial adhesions were noted in 4 patients, in one subject however, they were associated with melanosarcomatous metastases in the region The heart weights and myocardial changes in the adult material are given in table 1 where it is apparent that 50 hearts were devoid of major pathological features

Seven of the new born infants were full term and the eighth was a premature weighing 1100 g (II) None of the

Table 1 Heart weights and myocardial changes

Weight	Recent infarction	Recent and old infarct	Old infarcts	Scattered fibrosis	Myocardium without major changes	Total
—300	1			1	21	23
300—399	3	1	3	4	29	40
400—499	5	2	3	2	21	33
500—	1	4	5	5	13	[illegible]
Total	10	7	11	12	84	124

infants had lived for more than 2 days Two infants died during delivery, and a third from a heart anomaly (valvular aortic stenosis) The main diagnosis for the remaining five were intracranial hemorrhage (3) and pulmonary lesions (2)

Injection procedures

The bronchial arteries were injected with different techniques in the two series (II and IV) In the former (II) the bronchial arteries were catheterised after the aorta had been opened and a selective angiography was performed In the other series (IV) and in the new-born infants (II) the bronchial angiography was indirect The aortic arch, all the intercostal and brachiocephalic arteries including the internal mammary arteries were ligated, and injection was performed into the isolated aorta, the bronchial and other smaller mediastinal arteries being the only intact vessels leaving the aorta

The internal mammary artery was selectively injected (III) before the body cavities were opened

The injection material was an aqueous suspension of microcrystalline barium sulphate (Micropaque) The concentration of the barium suspension differed for the different series—25 per cent and 7 5 per cent in III and IV and 25 per cent with 3 per cent gelatine added in II With the pressure retort described by Ljungqvist (1963) the injection pressure was kept constant at a maximum of 120 mm Hg (II and IV) or 130 mm Hg (III)

The amount of contrast medium was kept constant at 50 ml when the internal mammary artery was injected In this procedure the amount of contrast medium could be standardized as the injection system was closed, e g a ligature could be put around the injection stalk and leakage was negligible The bronchial artery injection, however, could not be performed in a closed system and the amount of contrast medium could thus not be fixed Injection was terminated when contrast medium had been visible on the pleural surfaces for about ten minutes In the selective bronchial artery injection (II) the amount of contrast medium varied between 10 and 40 ml and in the other bronchial artery study (IV) the amount of contrast medium was about 500 ml If leakage had been excessive the amount of contrast medium was increased

For the newborn infants the contrast medium was a 7 5 per cent barium suspension, the injection lasted for 45 minutes and about 50 ml of contrast medium was used for each specimen (II)

Radiographic procedures

Radiograms of the injected specimens were obtained at 72 kV and 12 mA using Gevaert Osray films without intensifying screens and an exposure time that varied from 0 5 to 3 5 sec The focus film distance was 1 5 m and several projections were chosen to disclose contrast filling of arteries in the heart pericardium and lungs (II III and IV)

For microangiography (IV) exposures

were made with a Machlett OEG X-ray tube at 40 kV and 8 mA on Kodak maximum Resolution Plates (resolution at least 1000 lines/mm) To obtain a stereo-pair, each block was exposed twice and the blocks were moved from the vertical axis between the exposures to give a viewing angle of 9° The stereo-microradiograms were examined in a stereoscopic viewer, Sterant (NIFE) at a 7 × linear magnification Vessel diameters were measured microscopically on the microradiograms in regions where the vessels seemed to lie parallel to the photographic plate The optic system was a 6 3 × objective, N A 0 16, and an 8 × eye piece with an ocular micrometer that was calibrated against an object micrometer with 10 μ intervals

Quantitative evaluation of coronary stenosis

To find out whether there is a correlation between extracardial anastomoses and the severity of coronary atheromatosis, the degree of stenosis of the coronary arteries was determined according to the method described by Lober (1953) This involved microscopical examination of five transverse sections from the first five cm of each of the three main coronary arteries The cross-section area of the lumen is given as a percentage of the cross-section area of the entire artery using the outer external elastic lamina as the reference The greatest degree of stenosis was taken as the value for the coronary branch in question (II, III and IV)

Discussion on material and methods

The material (II, III and IV) was collected consecutively and there is a dominance for the older age groups and the new-born period as in most hospital autopsy series (cf Bjurulf et coll 1967) The low mortality in younger age groups reduces the possibilities of getting a uniform age distribution The main object with these studies was to determine the frequency and the size and shape of the extracardial anastomoses and this can—besides age—be influenced by heart weight, stenosis of the coronary arteries or a disease that may have occurred years before the actual examination Since some of these factors vary within the same age group an uneven age and sex distribution does not in itself influence the representativity of the material to a great extent

Injection procedures

The bronchial arteries were injected with different techniques in the two series (II and IV) In the first series (II) —selective bronchial artery injection— the main object was to determine the frequency of extracardial anastomoses This could best be done when relatively small amounts of contrast medium was injected and progress in the injection procedure followed by the naked eye

In the second series of bronchial artery injection (IV) the main object was to determine the size and shape of the anastomotic pathways There are usually three bronchial arteries and the branches emanate from different positions in

the descending thoracic aorta (Cauldwell et coll 1948) In the first series (II) it was found that some of these vessels were too small to be catheterised but were recognized when injection in a large bronchial artery resulted in a retrograde flow out into the aorta In order to get contrast filling of all bronchial arteries the injection was performed through the intact aorta—all irrelevant vessels were ligated so that the bronchial and other smaller mediastinal arteries were the only vessels leaving the aorta

In the initial series (I) it was found that in a few hearts one vessel was clearly outlined in the first radiogram but the same vessel was less evident after handling the specimen Some contrast medium was obviously lost during the manipulations with the fresh autopsy specimen This can be overcome either by formalin fixation of the thoracic organs or by modifying the contrast medium Fixation in formalin would—at the best—result in a stiff heart, which could be difficult to handle in further radiographic procedures The other recourse is to change the composition of the contrast medium

For selective injection into the bronchial arteries (II) the contrast medium was an aqueous 25 per cent barium sulphate suspension with 3 per cent gelatine added The amount of gelatine was chosen to facilitate solidification of the contrast medium at room temperature after being injected at about 40°C The autopsy specimens were kept at room temperature since a lower temperature would induce solidification of the contrast medium too prematurely to result in a contrast filling of only the first parts of the bronchial arteries

In the other study with bronchial artery injection (IV) the object was to get as near a total outline of the arterial system as possible The contrast medium with gelatine has a rather high viscosity and would not fill vessels of about precapillary size A 7 5 per cent aqueous barium sulphate suspension was therefore chosen since this concentration has proved suitable in other investigations in this institution (Ljungqvist 1963 Robertson 1967)

The amount of contrast medium needed for good contrast filling of the arterial system depends on the volume of the vascular system, leakage and anastomotic pathways The ideal vascular system would have only one artery that could easily be ligated after catheterisation and ordinarily no anastomoses to other vessels Such a vessel is the internal mammary artery and in that series (III) no major problems arose The bronchial arteries differ greatly from the ideal There are usually three arteries and due to the tortuosity at their origins the injection stalks cannot be ligated Furthermore, the arteries anastomose with the pulmonary and intercostal arteries and anastomose or have branches to arteries that supply the oesophagus The bronchial artery system is said to be totally outlined with about 10 ml of contrast medium (Schoenmackers 1959), but in the present studies it was found impossible to be certain of the amount of contrast medium that passed into the bronchial artery system and the amount

that was lost It was found impossible to set a limit for the amount of contrast medium needed for an adequate contrast filling The amount of contrast medium used ranged between 10 and 40 ml in series II and was about 500 ml in series IV The difference in the amount of contrast medium is more apparent than real if it is remembered that in series IV (i) the contrast medium had lower concentration, (ii) the dead space —the aorta—of the contrast column was greater and (iii) the better contrast filling of the bronchial arteries as well as the broncho-pulmonary anastomoses Furthermore, it has been suggested (Ljungqvist 1963) that with this type of contrast medium (IV) the water in the contrast suspension flows through the vessel walls and out into the tissues leaving only the barium granules of the medium in the lumen This means that a greater volume of contrast suspension is required to obtain the same relative degree of contrast filling

Radiographic procedures

The prime value of post-mortem angiographies is the demonstration of the course of the vessels and their anastomoses Caution is warranted in drawing conclusions about the calibre of the vessels and a failure to demonstrate an anastomotic pathway might well be due to the inherent difficulties in the angiographic method

In the microangiographic study (IV) reference was made to the width of the vessels The microangiographic and optic systems were standardised to give only negligible differences in the measurements between the specimens Allowance cannot be made, however, for the different degrees of distension of the vessels which possibly may be of minor degree since the measured vessels were anastomoses in an open injection system The values obtained for the vessel diameters are to be considered as mutually relative figures and cannot be related to figures from angiograms on living subjects

Quantitative evaluation of coronary stenosis

The present method of determining coronary stenosis could give similar relative figures for an arteriosclerotic lesion that develops during a long period of time and a ruptured atheromatous plaque or a mural non occluding coronary thrombus These lesions differ in their clinical significance and in the development of collaterals It is well-known that longstanding, slowly progressing lesions favour the formation of a collateral network and are correspondingly clinically favourable The present method of determining coronary stenosis—as in any post-mortem classification —reflects a static post mortem condition and gives only relative indications of the functional significance during life

Bronchial artery anastomoses

The initial study (I) demonstrated the existence of anastomoses between the bronchial and coronary arteries. With a standardised technique it was demonstrated (II) that such anastomoses were present in all subjects examined, provided that a successful injection was possible. The series consisted of 37 adults and 6 new born infants and obvious artefacts occurred in 7 injections of which 6 were in the adult group.

In series IV anastomoses between the bronchial and coronary arteries were demonstrated in all subjects, 38 adults. The indirect injection technique and the greater volume of contrast medium contributed to making all the injections successful.

Thus if obvious artefacts could be avoided, anastomoses between the bronchial and coronary arteries were demonstrated in 69 adults and 7 new born infants, the smallest a premature weighing 1100 g. Furthermore, vessels in the pericardium were filled with contrast medium in all specimens. These vessels were not only visible in the dorsal part but quite often in the anterior and diaphragmatic portions of the pericardium as well.

A major blood supply from an extracardiac source to a ventricular coronary artery must pass via the atrial arteries. These arteries were studied with stereoscopic microangiography (IV). In 5 of the 6 new born infants examined the arteries had a straight course and in the sixth a single spiral artery was identified among the straight arteries. Straight vessels were also visible in an 11-year-old girl and a 49-year old man. Spiral arteries were observed in the remaining 36 subjects. The largest spiral artery had a maximum lumen diameter of 880 μ, this patient was an 83 year old man with a 10-year history of repeated myocardial infarctions. Spiral arteries with a lumen diameter of more than 500 μ were noted in 8 subjects and seven of them were over 70 years of age. Vessels with a diameter of less than 200 μ were observed in 8 subjects, six of these were under 60 years of age. The lumen diameter of the largest spiral artery detected in each specimen seemed to increase with age.

The number of spiral arteries in each heart differed greatly between the specimens but appeared to increase with the age of the subject. The number of spiral arteries seemed to be greatest in specimens with severe coronary stenosis and extensive myocardial changes. An objective evaluation of this point was not made since a prerequisite for such a count is complete contrast filling of all vessels in each specimen. This can hardly be achieved by post mortem injection (Bellman 1953).

The lumen diameter of the largest spiral artery in each heart in the adult material was considered in relation to age, heart weight and the maximum degree of stenosis of the coronary arteries (conventional correlation analysis and the t test). Significant correlations were noted between the diameter of the

largest spiral artery and age (***), the maximum degree of coronary stenosis regardless of the artery affected (**) and the degree of stenosis in the left descending artery (**) Correlation between the largest spiral artery diameter and heart weight, the degree of stenosis in the right and left circumflex branches did not reach a significant level ( )

Internal mammary artery anastomoses

The results of the preliminary series (I) were similar to those of series III in which the internal mammary arteries were successfully injected in 49 subjects Anastomoses to the coronary arteries at the ventricular level were demonstrated in 4 subjects, in an additional 15 specimens contrast filling was noted in minor atrial arteries and in the remaining 30 hearts no contrast filling could be noted The pericardiophrenic arteries were clearly visible in 33 specimens and not visible in 2 An artery probably the pericardiophrenic artery but possibly a pericardial vessel, was seen in the remaining 13 specimens (one radiogram was under exposed and could not be used for this purpose) The data from subjects with contrast filled vessels in the heart—at the ventricular or atrial level—were compared with each other and with the rest of the material There was no apparent reason—age sex degree of coronary stenosis, heart weight myocardial changes or interval between death and autopsy—to explain the capriciousness in the demonstration of the anastomoses from the internal mammary arteries Furthermore vessels in the pericardium were filled with contrast medium in all specimens The vessels were visible not only in the anterior part but quite often in the dorsal and diaphragmatic portions of the pericardium as well

Discussion—relative frequency of bronchial and internal mammary anastomoses, significance of spiral arteries and their correlation with age and coronary stenosis

Anastomoses between the bronchial and coronary arteries were demonstrated in all subjects examined—adults as well as new-born infants—provided the injection was successful In this respect the results accord with those of Hudson Moritz & Wearn (1932) who found anastomoses between the coronary arteries and mediastinal vessels in the 31 subjects examined However they injected through the coronary arteries and the two series are not fully comparable On the other hand Petelenz (1963, 1965 a, b, c) found anastomoses between the bronchial arteries and the coronary arteries at the ventricular level in only 14 of 100 adult specimens and in an additional 26 hearts contrast medium "covered (to different degree) the auricular surface To compare the differences and similarities in the results is difficult since Petelenz reports lack information about the amount of injected contrast material Too small amounts of injected material would lead to an inadequate contrast filling of the vascular tree and would give too low a frequency for extracardial anastomoses

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 486

INVESTIGATIONS IN HAEMORRHAGIC DISORDERS WITH PROLONGED BLEEDING TIME BUT NORMAL NUMBER OF PLATELETS

WITH SPECIAL REFERENCE TO PLATELET ADHESIVENESS

BY

STIG CRONBERG

MALMÖ 1968

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I. *Cronberg S, Nilsson I M and Gydell K.* Haemorrhagic thrombocythaemia due to defective platelet adhesiveness. Scand J Haemat. 2 208—219 1965

II. *Cronberg S, Nilsson I M and Silwer J.* Studies on platelet adhesiveness in von Willebrand's disease. Acta med scand *180, 43—54* 1966

III. *Cronberg S, Robertson B, Nilsson I M and Nilehn J E.* Suppressive effect of dextran on platelet adhesiveness. Thrombos Diathes haemorrh (Stuttg.) *16* 384—394 1966

IV. *Cronberg S, Nilsson I M and Zetterqvist E.* Investigation of a family with members with both severe and mild degree of thrombasthenia. Acta paediat scand. 56 189—197 1967

V. *Cronberg S.* Methodological problems in testing of platelet adhesiveness. Scand J Haemat 4 385—400 1967

VI. *Cronberg S and Nilsson I M.* Investigations in a family with thrombasthenia of moderately severe type with 16 affected members. Scand J Haemat. 5 17—25 1968

VII. *Cronberg S and Nilsson, I M.* Investigations of patients with mild thrombasthenia — a haemorrhagic disorder with prolonged bleeding time probably due to a primary platelet defect. Acta med scand in press

VIII. *Nilsson, I M and Cronberg S.* A severe haemorrhagic disorder with prolonged bleeding time due to a plasma defect but with normal factor VIII. Acta med Scand in press

IX. *Cronberg S.* Effect of fibrinolysis on adhesion and aggregation of human platelets. Thrombos Diathes haemorrh (Stuttg.) in press

A study of deficient primary haemostasis
is a study of cellular and humoral pathology

When a blood vessel is injured, platelets adhere to the damaged vessel wall Other platelets then stick to the adherent platelets to form platelet aggregates which finally fill up the vessel This platelet mass is first loose but subsequently consolidates into an impermeable plug (122)

This formation of a platelet plug is the primary stage of haemostasis The underlying mechanism is not properly understood, but various schemes (Fig 1) have been put forward as working hypotheses (207, 152) All these hypotheses assume that adhesion of platelets to the collagen fibres of connective tissue is of primary importance (33, 123, 124, 125, 270, 121, 243) Adenosine diphosphate, which is present in red cells and platelets induces aggregation of platelets (108, 86, 30) It is released from the platelets themselves after contact with connective tissue (121, 243), on exposure to thrombin (136, 97), or to other stimuli ADP therefore probably plays a fundamental role in platelet aggregation Calcium ions and plasma factors, such as the von Willebrand factor (183, 224) and fibrinogen (101 71 160) may be of importance Thrombin formed by the coagulation factors consolidates the primary platelet plug and induces viscous metamorphosis and clot retraction (268, 269, 151, 152) It also induces platelet aggregation, but the physiological importance of this observation is debatable (p 28)

Defective primary haemostasis results in a haemorrhagic diathesis with bleeding from mucosal membranes, with menorrhagia, ready bruisability, or obstinate bleeding after surgery The most conspicuous laboratory abnormality of this type of haemorrhagic diathesis is a prolonged bleeding time, and the syndrome is found in a variety of congenital and acquired conditions, of which thrombocytopenia is by far the commonest It also occurs in patients with primary platelet defects, such as various kinds of thrombasthenia (pp 18—21) thrombopathy and haemorrhagic thrombocythaemia (p 23), plasma defects such as von Willebrand s disease (p 15), and a related but different disease (p 17), in uraemia (p 23), macroglobulinaemia Waldenstrom (p 23), after administration of large amounts of dextran (p 25), and after induced fibrinolysis (p 24)

The aim of this study was to investigate the laboratory and clinical features in patients with haemorrhagic disorders characterised by a prolonged bleeding time but with a normal platelet count Special attention was given to the adhesiveness of the platelets and investigation of various factors capable of influencing the results (pp 8—13)

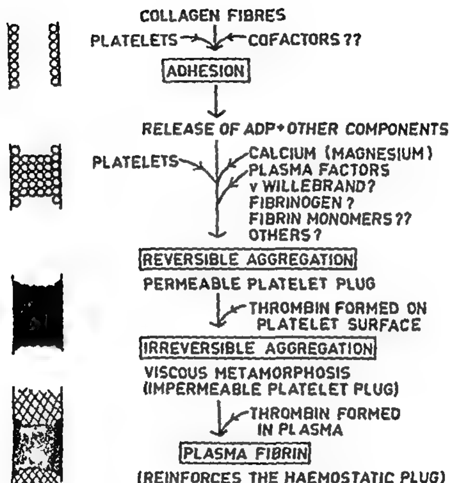


Fig. 1. Schematic illustration of the primary haemostasis. Modified from a scheme by Owren (207). Many reactions are still obscure and debatable.

METHODS

Clinical investigation

A careful inquiry was made into the patients histories They were asked whether they bruised easily, whether they suffered from nose bleeding, gingival bleeding, menorrhagia, urinary or gastro-intestinal bleeding, joint bleeding, and whether they had bled obstinately after surgery or tooth extraction or at parturition

The familial history was thoroughly inquired into since many bleeding disorders have a typical hereditary pattern Notes were also made of any unrelated conditions, such as pregnancy and various systemic infections and metabolic diseases as well as various drugs, such factors often being capable of influencing the clinical and laboratory findings

Laboratory studies

Bleeding time

Method of Duke Using standardized haemolets (Dade Reagent, Inc, Miami, Florida, USA) determinations were made on both ears Normal range 1 to 4 minutes

Method of Ivy The method of Ivy was used as modified by Borchgrevink and Waaler (25) and also described by Nilsson et al (189) An arm cuff was placed on the upper arm and inflated to 40 mm Hg On the volar side of the forearm 3 transverse incisions, 1 mm deep and 10—14 mm long, were made with a surgical blade (Gillette Surgical Blade F) Every 30th second the blood shed was gently absorbed with a filter paper until the bleeding stopped The mean of the three determinations was taken as the patient's bleeding time The overall mean of such triplicate determinations in 35 normal individuals was found to be 9 5 minutes (range 5—15 5 minutes) (189) Later investigations in the course of this study have given similar results and in 70 normal individuals the mean was found to be 10 0 minutes with a range of 5—20 minutes, standard deviation ± 3 1 and standard error ± 0 37 The bleeding time exceeded 15 minutes in two out of the 70 individuals

Comments The Duke bleeding time is prolonged only in patients with severe haemorrhagic diathesis, and a prolonged Duke bleeding time contraindicates surgery (189) The Ivy bleeding time is more sensitive and often exceeds 30 minutes in patients with mild thrombasthenia and mild von Willebrand s disease This method is therefore the best for detecting such conditions (189) In the range of 13—20 minutes the values found in the volunteers and the patients overlapped In such individuals all doubt was usually dispelled by repeated investigations and other clinical and laboratory signs

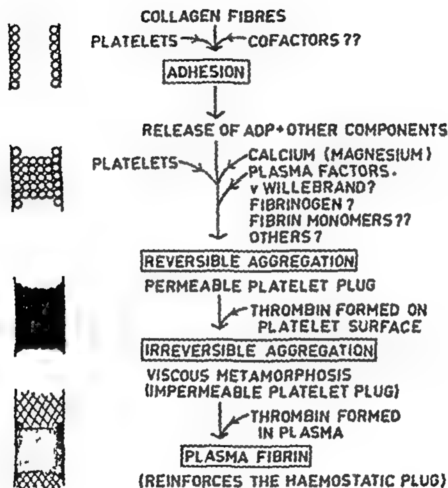


Fig. 3. Schematic illustration of the primary haemostasis. Modified from a scheme by Owren (?0?). Many reactions are still obscure and debatable

ween adherent and free platelets The investigations were performed on ordinary glass slides and glass slides siliconised with a 5 % solution of Siloxan (Uddeholm, Sweden) in chloroform and heated for 2 hours at 160°C temp

For studying the spread and aggregation of platelets after minor trauma Breddin developed two techniques For studying platelet-spread a drop of platelet-rich citrated plasma diluted with a mixture of physiological saline and citrate is placed on a plastic slide and the platelets then sediment and adhere and spread out on the slide, and after 30 minutes the reaction is stopped by immersion in 10 % formol (37) The smears are then stained with Giemsa stain The apperance of the platelets is graded according to degree of spreading In his aggregation test (PAT) platelet-rich citrated plasma is rotated for 10 minutes in a siliconised glass bulb and is then diluted and placed on a plastic slide for 30 minutes to allow the platelets to adhere to it, after which the preparation is fixed with formol and stained with Giemsa solution (36) The degree of platelet aggregation and fusion is noted and graded in five steps *

Platelet aggregation and viscous metamorphosis were also studied in platelet-rich citrated plasma after recalcification addition of thrombin and other substances A drop of platelet-rich citrated plasma was placed on a glass slide under a cover-slip The drug to be investigated was then added from the side, and the platelet reactions were observed in the phase contrast microscope

Platelet aggregation was also studied by the photometric technique (p 12)

* With these techniques several of our patients were investigated by a member of his staff (Dr I Scharrer) which is gratefully acknowledged

Adhesiveness tests**

Hellem s whole blood method

Hellem s original method (108) was used with just a few minor changes (67, 60) Citrated whole blood was passed through a glass bead filter at a constant rate The numbers of platelets present before and after the passage were compared and, unless otherwise stated the result was expressed as a percentage of platelets retained by the glass beads The minor deviations from Hellem s original description were, first, in order to maintain a transit time of 30 seconds with our utensils, we had to use aggregates with 4 6 g glass beads instead of 5 g and, second, the test was mostly performed one hour after sampling

Personal experiments with Hellem's method (60)

A mathematical approach demonstrated that the interstitial volume in a column with equal sized, spherical glass beads was not dependent on the size of

** According to the recommendation of the International Committee on Haemostasis and Thrombosis adhesion should mean the sticking of platelets to a surface other than a platelet, and sticking of platelets to one another should be called aggregation. As many or most of the platelets in the adhesiveness tests will in reality stick to one another the term adhesiveness test is generally inadequate It has, however been in common use and we therefore continue to use it when there can be no misunderstanding fully recognising that not all the retained platelets in reality adhere to the glass surface.

the glass beads The total surface area was inversely proportional to the radius of the glass beads The mean height (thickness) of the layer of fluid over the surface was estimated by division of the interstitial fluid volume by the total surface area Other parameters, such as the mean circumference of the glass beads in a cross-section of a column and the length of glass surface the platelets had to pass over, could also be estimated

Experiments were performed with columns of various lengths and breadths, contact times, rates of flow, sampling times, size and nature of the glass beads The platelet contact time, *i e*, the transit time of the platelets, was found to be the most important parameter and the platelets were found to be retained in an exponential way (60) The mean height of the fluid modified the results at low rate of flow but was less important when the rate was increased Otherwise adhesion was influenced only little by variation of the parameters of the column Thus the length varied from 14 cm to 64 cm with little change in the number of platelets retained when the platelet contact time (transit time) was kept constant at 30 seconds Accordingly the total glass surface area was also of less importance It must be remembered however, that when blood was collected in fractions the number of retained platelets increased from fraction to fraction This was found to be due to accumulation of an enhancing principle and this was favoured by the use of small glass beads long platelet contact time long contact of blood to the glass surface and long length of the column Further experiments suggested that the enhancing principle was the R-factor of Hellem (108) released from injured red cells and later identified as ADP (86)

Variation of the temperature has but little effect Higher citrate concentrations abolish the adhesion and aggregation In a patient with low haema tocrit the citrate concentration will be lower owing to higher dilution This decreased concentration was found to have but little influence, and therefore the same proportion of 9 parts of whole blood and 1 part 3 8 % sodium citrate dihydrate was used throughout Optimal and rather constant values were ob tained when the blood was tested about one hour after sampling This time was therefore used

The mean found for 58 normal persons, mostly 20—30 years of age, was 30 0 with a standard deviation of ± 3 6 and a standard error of ± 0 8 (67) The adhesiveness was found to be higher in the aged in 20 normal, healthy" persons of both sexes about 80 years of age the mean adhesiveness was 43 8 % with a standard deviation of ± 11 1 and a standard error of ± 2 5

To summarise, Hellem's whole blood method has many well standardised parameters The adhesiveness was normally 30 %, which was valuable for detecting patients with increased adhesiveness The dimensions or composition of the columns were satisfactory but a higher normal value might have been more suitable for detection of lowered adhesiveness

Hellem's plasma + ADP method

Hellem's original description (111) was followed with but few modifications (67). ADP in low concentration was added to platelet-rich plasma, mixed, and then immediately passed through a glass bead column. The disadvantage of the method was that ADP had to be added at a critical low concentration (final concentration 0.1, 0.05 μg/ml), at which factors influencing ADP degradation play a great role (205, 236, 245). Thus the plasma temperature and the interval between addition of ADP and passage of the mixture through the column must be strictly standardised (245). With increased concentration of ADP the platelet adhesiveness was always high except in Glanzmann's severe thrombasthenia. The error of the method was large.

Our own mean values based on 63 normal persons, about 20—30 years of age, was 48.1 % at an ADP concentration of 0.1 μg/ml with a standard deviation of ± 14.8 and a standard error of ± 2.1. The mean adhesiveness at an ADP concentration of 0.05 μg/ml was 25.9, s.d. ± 12.3, s.e. ± 1.6.

Salzman's method

The original method of Salzman (224) was used with a thin needle. Fresh blood was drawn directly from a vein through a short column of glass beads into a vacuum tube with EDTA. The tube should be filled with about 5 ml in 40—50 seconds (60).

Calculations and experiments with Salzman's method (60)

With Salzman's method a rapid blood flow is achieved and the platelets pass through the column in about 3-4 seconds (60). As will be apparent, the rate of flow is not constant during the sampling and also varies from one occasion to another.

The 8 ml Vacutainer© contains a small amount of air already at the start. At atmospheric pressure we have found that every tube contains about 1.5 ml of air. To this may be added 0.3 ml of air that is present within the filter at the start and will be transferred to the tube. When the blood starts entering the tube, the latter will contain 1.8 ml corresponding to a pressure of 171 mm Hg. The suction pressure will be the atmospheric pressure plus the venous pressure minus the pressure inside the tube. According to physical laws, other conditions being constant, the rate of flow will be directly proportional to the pressure differences. As the tube is filled, the air inside the tube will gradually occupy a smaller volume and its pressure will increase. The rate of flow will then decrease. This is slight during the flow of the first few millilitres and most of the blood will therefore ordinarily pass through rapidly with no overt difference in rate. However, when 4 ml blood has entered, the rate will have decreased to 71 % of the initial value. More important, then, is that the initial rate may vary. This will depend on the viscosity of the blood and the resistance of the system and the position of the tip of the needle in the vein. Assuming that the tube receives 4 ml in 50 seconds and 6 ml in 45 seconds, the initial rate will vary from 180—360 cm/min and

the platelet contact time, at the start from 3 3 to 1 7 seconds In anaemic patients with low blood viscosity the tube will often be filled too rapidly Towards the end the rate of filling becomes increasingly lower, and it is *therefore left to the discretion of the* examiner to decide whether the tube is filled at 40 seconds or less and thus to accept or reject the sample

If the column is made half as long, the rate will increase and the tube will be filled within 30 seconds, and the platelet contact time will be very short owing to difference in length and the rapid flow Accordingly, if the column is made double the ordinary length the tube will hardly be filled within 60 seconds and the platelet contact time will be much longer The differences in filling time can, however, be compensated by the use of needles of different diameters Experiments have revealed that adhesion is very poor or absent in the shorter columns and much stronger in the longer ones, while most normals will have values between 25—40 % with columns of ordinary length (60) Our own mean found for 34 healthy normals about 20—30 years of age was 33 % s d ± 17 0 s e ± 2 9 Recently Salzman recommended columns with 1 3 g glass beads instead of 1 0 (226) In our latest investigations these were used in parallel and gave somewhat higher values

Empirically Salzman (224) found the adhesiveness in von Willebrand's disease to be low as measured with this method Our own results are reported on p 15 and p 35—37

Aggregation of platelets

Platelets were examined for aggregation by various methods, *viz* by direct observation in the test tube, by observation on a glass slide in the phase contrast microscope (p 8), by the method of *Breddin and Bauke (36) (p 9)*, and also by a photometric technique using a modification of the method of Born (28) Platelet-rich plasma was placed into a cuvette and the optical density read in a Linson Photometer (Ljungberg & Co, Stockholm, Sweden) at a wave length of 620 μ The substance to be tested was then added and the cuvette shaken by hand and the optical density was read at short intervals The optical density of platelet-free plasma was recorded The aggregation activity was expressed by converting the actual fall in optical density produced by the test solutions to a percentage of the reduction in density produced by spinning the plasma at 2,000 *g* for 10 minutes

In many experiments estimations were made of the effect of various substances on the aggregation of platelets in platelet-rich plasma after addition of ADP (final concentration about 1 μg/ml) or connective tissue suspension

Comments Photometric methods for studying platelet aggregation have been widely used by physiologists and pharmacologists under various experimental conditions (28, 30, 197 and others) Photometers permitting continuous stirring and automatic recording are preferable Opinions differ concerning the interpretation of the curves (201, 203) No close correlation between aggregation and glass adhesiveness with the

plasma-ADP method was found by Rozenberg and Stormorken (222) In the severe thrombasthenia of Glanzmann's type addition of even a large dose of ADP will not result in any aggregation (68) In von Willebrand's disease Vainer and Caen (251, 252) reported a slight abnormality Slight abnormalities have been reported also in other conditions (p 21)

Clot retraction

Clot retraction was investigated by a modification of Voss method (253, 68), according to which dilute platelet-rich plasma is coagulated with thrombin and the clot retraction measured at various intervals

Comments Under these experimental conditions clot retraction readily occurs and abnormal reactions are noted only in patients with severe thrombasthenia of Glanzmann's type or with thrombocytopenia Normally, the clot had shrunk by 70 % within 3 hours so that the length of the clot then was 30 % of its original length Bettex-Galland and Luscher (14) studied the metabolism of the platelets during clot retraction and stressed the significance of ATP and glucolytic activity in this reaction

Platelet factors 1, 3 and 4

Platelet factor 1 (= factor V activity), platelet factor 3 (= thromboplastic activity), and platelet factor 4 (heparin binding capacity) were determined as described by Nilsson et al (193)

Comments The platelets are carefully washed, finally in distilled water, and frozen Despite this crude treatment the factors are preserved, but many membranes are opened up and differences if any, in the physiological availability of the factors might not be demonstrated (154)

Coagulation time

This was determined in glass tubes (184) It was also determined in plastic tubes which were allowed to stand and examined every minute The normal range in this laboratory was 8—15 min in glass tubes and 15—25 min in plastic tubes

Prothrombin consumption test

This test was performed according to Biggs and Macfarlane (17) The values found for normals in our laboratory usually ranged from 0 to 30 %, occasionally somewhat higher

Coagulation factors

The AHF (factor VIII) activity of plasma was assessed by its normalising effect on the recalcification time of platelet-rich haemophilia A plasma with < 1 % AHF (182, 184 185) and the amount of AHF present was expressed as a percentage of that found for a normal standard consisting of pooled plasma from 10 individuals

Haemophilia B-factor (f IX) was tested in a similar system with f IX deficient plasma

Factor V, factor IX, fibrinogen, prothrombin + factor VII + factor X (Owren's P&P-test), thrombin time, recalcification time and test for circula-

ting anticoagulant were determined as described earlier (184) In many patients PTA (factor XI) was determined with a modified TGT-test (177)

Comments This method for testing factors VIII and IX by studying the ability of the patient's plasma to correct deficiency plasma proved more sensitive and accurate than the TGT method (186) With that method slight decreases in factor VIII to 50 % often escaped detection, whereas a serum defect often falsely was found, suggesting deficiency of factor IX or XI

Investigation for fibrinolysis

Euglobulin clot lysis time and *fibrinolytic activity of* plasma and resuspended euglobulin precipitate on unheated and heated bovine fibrin plates were determined as has earlier been described (191, 192)

Fibrinolytic split products were identified and quantitated by immunological methods (172, 173)

Fibrinogen was determined by a modification of Jacobsson's method as described by Nilsson and Olow (191)

CLINICAL INVESTIGATIONS

I CONGENITAL DISORDERS

1 *von Willebrand's disease*

This is the haemorrhagic disorder of the patients on the Åland Islands first described by von Willebrand (*259, 260*) and is characterised by a prolonged Ivy bleeding time, a low factor VIII and a dominant hereditary pattern (180)

In 1956 and 1957 Nilsson and co-workers described 13 Swedish patients with an inherited autosomal dominant *haemorrhagic diathesis characterised by* AHF (factor VIII) deficiency and prolonged bleeding time (182) In these patients the platelets were normal in respect of platelet factor 3 In 1953 to 1956 other authors reported 32 instances of the same type of bleeding disorder (reviewed in 180) Nilsson et al (179) found that it was possible to correct not only the AHF deficiency but also the prolonged bleeding time and capillary *bleeding by injecting AHF containing* fraction I—O prepared aseptically by the glycine method of Blombäck and Blombäck (23) In 1956 a patient was subjected to hysterectomy under cover of fraction I—O without any abnormal loss of blood at operation (179)

The clinical features of the Swedish patients resembled those of von Wille brand s disease in Åland but the finding of a normal platelet factor 3 was not compatible with such a diagnosis (134) In 1957 fifteen patients with von Willebrand s disease and living in Åland were investigated In all of them the AHF was found to be decreased and platelet factor 3 normal (183) One of the patients responded favourably to infusion of human fraction I—0 and the conditions were therefore identical

Fraction I—0 prepared from haemophilic A plasma also corrected the bleeding time and produced an unexpected increase in factor VIII This indicated *that the prolonged bleeding time* was caused by lack of a plasma factor present in haemophilic plasma and therefore not identical with factor VIII (181)

The results obtained by Nilsson et al have since been confirmed by other investigators (58, 59 and reviewed in 119)

According to Nilsson and Blombäck (180), two types of patients with von *Willebrand s diesease can be recognised* severely affected patients with a Duke bleeding time exceeding 20 minutes and a factor VIII content of 1—20 %, and mildly affected patients with a normal or slightly prolonged Duke bleeding time in the range of 2—20 minutes but a prolonged Ivy bleeding time exceeding 15 minutes and a decreased factor VIII in the range of 10—60 % The severity varies both between different members of the same family and also *periodically* in one and the same patient Factor VIII is practically always low, but occasional-

ly it may be normal, as in pregnancy In doubtful cases the effect of administration of fraction I—0 or fresh plasma can help to settle the diagnosis So far more than 200 cases of von Willebrand s disease have been diagnosed in Sweden and a survey is under preparation (233)

The exact mode of action of the von Willebrand bleeding factor is not known. Nilsson and coworkers (175) suggested that it might act on the capillary wall or on platelets or on both. Salzman (224) and Odegaard et al. (204) proposed that it was a necessary cofactor for platelet adhesion. Special investigations were therefore performed to test the platelet reactions in von Willebrand s disease

Investigation of platelet function in von Willebrand's disease

When investigated under direct observation with a phase contrast microscope the platelets in platelet-rich citrated plasma from more than 20 patients with von Willebrand s disease were found to adhere to the glass slide and aggregate in a normal way

When the platelet adhesiveness was tested with Hellem s whole blood method or plasma-ADP method in 68 patients with von Willebrand s disease adhesiveness was found to be normal (67) and an earlier report of a decreased adhesiveness in 5 patients (204) was not confirmed With Salzman s method we confirmed his results and those of others (224 161 162 246) and found the adhesiveness to be decreased in most patients (Appendix I p 35) In Salzman s method the blood is rapidly withdrawn directly from the needle through a filter into a vacuum tube (p 10) Low adhesiveness, as tested with Salzman s method, was also found in patients with thrombasthenia (65) and has been reported also in patients with uraemia (229) Low adhesiveness, as judged by Salzman s method was therefore not pathognomonic of von Willebrand s disease and the method could not substitute determination of factor VIII, but it was useful as a complement to the Ivy bleeding time — both being of equal dignity From a theoretical point of view, the experiences with Salzman s method are interesting Under the auspices of the International Committee on Haemostasis and Thrombosis a joint investigation has been performed to assess the value of Salzman s method in the diagnosis of this disease. We have taken part in this investigation and the results of our investigations are included in Table 3 in Appendix I p 36

Patients with von Willebrand s disease were found to develop atherosclerosis, and deaths from myocardial infarction were reported (234) Theoretical considerations on the von Willebrand bleeding factor are discussed on p 28

Treatment of von Willebrand s disease

The best treatment available is administration of fraction I—0 or fresh plasma (181 180 19, 20) or cold precipitate (8 210) This shortens the bleeding time and increases the factor VIII Epsilon-amino-caproic acid (EACA) suppresses the local fibrinolytic activity and thereby also bleeding after tooth

extractions (20) and in profuse menorrhagia Menstrual bleeding also responds favourably to gestagens (177) As in other bleeding disorders, at surgery all precautions must be taken to obtain satisfactory haemostasis

2 "Morbus Rita"

This disease was found in a young girl with severe bleeding symptoms It resembled severe von Willebrand s disease in that the Duke bleeding time was markedly prolonged but was shortened by plasma infusions (187) Platelet aggregation and clot retraction were normal but, unlike what is seen in von Willebrand s disease, factor VIII was always normal Platelet count and other coagulation factors were also normal and there was no pathologic fibrinolysis The bleeding time was shortened by treatment with fraction I—0 but no retarded increase in factor VIII was observed In contrast with what we have found in von Willebrand s disease, also stored plasma shortened the bleeding time, and better results were obtained when the plasma was prepared from donors of the same blood group as the patient The platelet adhesiveness as tested with Hellem s whole blood method bordered the lower limit of the normal range and was normal when judged with the plasma-ADP method The platelet adhesiveness, as tested with Salzman s method was repeatedly low As opposed to von Willebrand s disease there was no familial incidence of haemorrhagic disease and examination of her parents revealed no abnormality The patient was treated with plasma infusions and fraction I—0, and also responded favourably to epsilon-aminocaproic acid (EACA) (178) Her previously severe menorrhagia responded well to treatment with gestagens

It was concluded that this patient has a congenital haemorrhagic disorder related to, but definitely not identical with, von Willebrand s disease Theoretically, one might imagine the condition to be due to an isolated defect of the von Willebrand factor or of some factor activating this or activated by it We have not observed this condition in any other patient

3 Other conditions possibly related to von Willebrand's disease

A patient with a prolonged bleeding time and decreased factor XI (257) and a few patients with a prolonged bleeding time and low factor IX (B-factor) have been reported (241, 56, 100, 95, 235, 77, 21, 206) A similar patient reported by Gaston et al (88) was considered by them to have haemophilia B but with a coincidentally prolonged bleeding time We have never seen any patient in Sweden with a von Willebrand like disease with low factor IX instead of a low factor VIII We have investigated 4 patients belonging to two families reported to have such a syndrome but were unable to demonstrate the factor IX deficiency observed with the TGT-method using Nilsson and Blomback s method whereas factor VIII was found to be decreased The bleeding time of the patients was prolonged and the platelet adhesiveness was low, as tested with Salzman s method but normal with

Hellem's method In one of the patients the bleeding time was shortened on treatment with fraction I—0 from Kabi

4 Glanzmann's severe thrombasthenia

This disease is characterised by severe bleeding symptoms, a prolonged Duke and Ivy bleeding time absence of platelet aggregation after addition of ADP or other substances and a pathologic clot retraction

The term thrombasthenia was introduced by Glanzmann (92), who described patients with an increased tendency to bleeding and impaired clot retraction despite a normal number of platelets Formerly, before it was known that the AHF (factor VIII) is decreased in von Willebrand's disease, this disorder was often misdiagnosed as thrombasthenia In recent years patients with typical severe thrombasthenia were found to have platelets without any ability to adhere to glass or to aggregate after addition of ADP thrombin or other stimuli These characteristics clearly distinguish this condition from other types of haemorrhagic disorders and are usually considered obligatory for the disease (102, 43) A decreased availability of platelet factor 3 on incubation of platelet-rich plasma with kaolin has been reported (49, 102)

Cronberg et al (68) investigated a family with 3 affected members two sisters and one female cousin The Duke bleeding time usually exceeded 30 minutes The platelets were of normal shape and size but did not adhere or spread on glass or plastic surfaces In citrated platelet rich plasma the platelets did not aggregate after addition of ADP, connective tissue suspension, thrombin, papain or trypsin Thirty close relatives were investigated and nine of them were found to have moderate bleeding symptoms, prolonged Ivy bleeding time and a more or less decreased platelet adhesiveness It seemed likely that these were carriers of the gene for severe thrombasthenia, but in a single dose, while the severely affected patients had the gene in a double dose The ancestors could be traced back 6—9 generations and consanguinity between the parents of two of the patients was found 6 generations back This line did not include the maternal grandmother, who had the mild form and the investigation was therefore not quite conclusive In other reports consanguinity has been common (43, 212) As both mild and severe types have some features in common and are probably genetically connected we suggested the term severe thrombastenia for the classical severe type with complete inability of the platelets to aggregate, and the term mild thrombasthenia for the mild cases (p 20)

Treatment No specific treatment is available Platelet transfusions have been suggested (102) but we have found such treatment unsuccessful (68) Fresh plasma or fraction I—0 was without effect (68), but transfusions with fresh blood should be of value in bleeding states Prednisone might be of some value in the management of acute bleeding episodes and as in other haemorrhagic disorders in the event of surgery, special

precautions should be taken to secure effective haemostasis Epsilon-amino-caproic acid (EACA) which depresses *local fibrinolysis has been found* valuable in the control of bleeding after tooth extractions and in menorrhagia *Gestagens were found to be valuable to* decrease the amount of blood lost at menstruation.

5 Moderately severe thrombasthenia

This name was used to designate a bleeding disorder found in a large family with marked bleeding symptoms, a *prolonged Ivy bleeding time*, absence of spontaneous aggregation of platelets in platelet-rich plasma on a glass slide, decreased platelet adhesiveness, abnormal swelling of the platelets when suspended in citrate, and a dominant heredity (65)

The disorder was found in a family with sixteen affected members in 4 generations The bleeding symptoms were nose bleeding ready bruisability, menorrhagia, and obstinate bleeding after tooth extractions and surgery The bleeding symptoms were marked, but not so severe as in severe thrombasthenia of Glanzmann s type As many as seven of the members were genetically proven *carriers of the disease* The Ivy bleeding time was prolonged it was more than 30 minutes in fourteen of the patients and *16 respectively 17 minutes* in the other two The platelet adhesiveness as tested with Hellem s whole blood method, was more or less lowered The adhesiveness according to Salzman's method was low in nine out of thirteen Aggregation occurred after addition of ADP, thrombin or connective tissue suspension to platelet-rich plasma On suspension in citrate, especially when tested with Hellem's plasma-ADP *method*, the platelets of the patients assumed a swollen appearance Factor VIII was within *normal limits in all the patients* Prothrombin consumption test was definitely abnormal in four patients but normal in ten and bordered the normal limit in two A decreased availability of platelet factor 3 may account for the abnormal values in some patients, but the prothrombin consumption test gave results too divergent to be of diagnostic value When determined, platelet factor 3 was found to be normal One patient *received fraction I—O, which did not* shorten the bleeding time The bleeding symptoms, the prolonged Ivy bleeding time, the dominant hereditary pattern and the low adhesiveness with Salzman s method suggested von Willebrand s disease, but this diagnosis could be ruled out because of the different platelet reactions and the normal factor VIII The differential diagnosis is imperative because, unlike patients with von Willebrand's disease, these patients do not benefit from treatment with fraction I—O or fresh plasma In a therapeutical trial we infused a *fat emulsion, Intralipid®*, in two patients as we had found that it increased the retention of platelets in *Hellem s whole blood* test in normals (63) Intralipid increased the adhesiveness but did not shorten the bleeding time, we therefore concluded that this treatment was useless

The 83 year old woman, who was an ancestress of all the other fifteen pa-

tients, had had severe bleeding symptoms and shown typical laboratory findings, but had nevertheless developed severe atherosclerosis of the aorta and other arteries

We have found this special type of abnormality only in this family

6 Mild thrombasthenia

This diagnosis was made in patients with increased bleeding symptoms of the same type as in the aforementioned family, with prolonged Ivy bleeding time and with more or less decreased adhesiveness according to Hellem's whole blood method or Salzman s method and with no other demonstrable bleeding disorder that could account for the increased bleeding tendency (64) Aggregation of platelets in citrated plasma after slight traumatisation was generally weak, as judged by direct observation or by the PAT test of Breddin (64)

The disorder was found in more than twenty members of fifteen families and among close relatives of patients with severe Glanzmann s thrombasthenia The incidence was familial, but the heredity was not so obviously dominant as in the aforementioned family Siblings, one of the parents or their children often showed the same syndrome, but in some cases both parents were apparently normal Nose bleeding, bleeding after tooth extraction, menorrhagia and ready bruisability were the complaints that had most often prompted the investigation The bleeding symptoms varied in severity, but were occasionally severe and two patients had been hysterectomised because of menorrhagia and had later been referred for investigation because of other bleeding symptoms The bleeding symptoms in relatives with a similar syndrome were usually milder In one and the same patient the bleeding symptoms and the laboratory findings differed from time to time The abnormality observed in the relatives of the patients with severe thrombasthenia was indistinguishable from this condition (p 18) and the patients may have the gene for severe thrombasthenia in a single dose The disease is probably common but apt to escape detection, and the diagnosis is difficult One of our patients had first been considered normal despite a moderately prolonged Ivy bleeding time, but the condition was later recognised when the patient was referred to us becau e of other bleeding symptoms

Patients with a prolonged bleeding time as the only symptom have been described (128) In a survey of French patients with bleeding disorders Larrieu (146) reported 19 patients with a prolonged bleeding time as the only symptom, and these patients may belong to this group Hirsh et al (114) described a patient with spontaneous bruising where the investigation showed a prolonged bleeding time and complete absence of aggregation of the platelets after addition of connective tissue suspension This patient evidently differed from our patients in whom this test did not show any qualitative abnormality Other authors have described patients that may be closely related to, or identical with our patients Thus, Hardisty and Hutton (103) described 13 patients

with mild bleeding tendencies where a normally rapid platelet aggregation on addition of ADP was followed by unusually rapid disaggregation Platelet aggregation in response to addition of collagen suspensions *in vitro* was impaired and platelet adhesiveness to glass was usually decreased Weiss (254) has described a bleeding disorder in 6 women The disorder was characterised by a defect of platelet factor 3 availability and defective release of aggregating activity from the platelets on incubation with kaolin Under the name of Portsmouth syndrome O Brien (199) described ten patients with mild bleeding symptoms, prolonged bleeding time, decreased platelet adhesiveness and an abnormal reaction with a connective tissue suspension Hirsh et al (115) found it difficult to determine the normal range of the connective tissue platelet reaction because the activity of the connective-tissue extract varied from batch to batch and decreased on storage They therefore considered the reaction to be abnormal only when aggregation could not be demonstrated at all with a very active connective tissue extract and this is the view we have adopted The disease may therefore be closely related to, or identical with ours The patients of Hardisty and Hutton and of Weiss were regarded as belonging to the group of thrombopathy As the prothrombin consumption test was normal in most of our patients and platelet factor 3 when tested was normal, we hesitated to call the condition thrombopathy especially as a similar syndrome was present in relatives with Glanzmann thrombasthenia The difference in nomenclature should not be exaggerated and may be semantic and didactic rather than indicating a real difference

The condition is probably present in many patients with bleeding symptoms of unknown cause The treatment is the same as that for other patients with thrombasthenia

7 Dystrophie thrombocytaire hémorragipare congenitale" (Bernard and Soulier)

This is a well defined disorder characterised by bleeding symptoms, prolonged bleeding time and abnormally large, giant', platelets The disease has a recessive mode of inheritance but heterozygotes often show some abnormal platelets (135, 72) The disease was first described by Bernard and Soulier (13) and has since been reported by various authors (12 135, 72 99) The platelet counts are often moderately decreased No such case has been diagnosed in Sweden

In another congenital disorder the platelets are also large show abnormalities and are mostly decreased in number but abnormal leucocytes are also found (107)

8 Thrombocytopathy or thrombopathy

This is a primary platelet disorder characterised by bleeding symptoms, prolonged bleeding time and defective platelet factor 3 function

It is a rare condition and its classification is debatable In many patients there is a deficient availability of platelet factor 3 because prothrombin con-

sumption is abnormal, but the platelet factor 3 inside the platelets is normal (250) Other patients have the typical form with both abnormal prothrombin consumption and low platelet factor 3 (249, 132) Before it was known that factor VIII is decreased in patients with von Willebrand's disease these patients were referred to this group because of the abnormal consumption of prothrombin, but it is now known that platelet factor 3 is normal in this disease (180) In our patients with mild or moderately severe thrombasthenia we have occasionally found an abnormal consumption of prothrombin but no consistent changes

Other related or identical disorders have been discussed under the heading of mild thrombasthenia (p 20)

9 *Afibrinogenaemia*

These patients lack fibrinogen The bleeding time has been found to be moderately prolonged (101 126) The platelets lacked adhesiveness to and spreading on glass (101, 126) After addition of ADP in rather high concentration Gugler and Luscher (101) found that the platelets aggregated in a normal way, but other authors who used weaker ADP concentrations reported aggregation to be weak (238, 10, 126, 218) Small amounts of fibrinogen proved sufficient to correct the abnormality (101) Fibrinogen is therefore a necessary cofactor for adhesion of the platelets to glass and may be of importance also in other reactions (71, 160, 240) We have not investigated any patient with afibrinogenaemia, but some with hypofibrinogenaemia In these the adhesiveness of the platelets as judged by Hellem's whole blood method was usually normal One of them had a constant fibrinogen level of 0 02—0 07 g per 100 ml despite a pronounced and fatal predisposition to thrombosis (190)

10 *Other congenital disorders with prolonged bleeding*

This may occur in unrelated disorders without evidence of platelet or plasma defects Such bleeding may be seen in Ehlers-Danlos syndrome, which is caused by abnormal connective tissue and characterised by hyperextensible joints and extreme laxity of the skin The patients show a mild bleeding tendency, but the bleeding time is mostly normal We have investigated one such patient who was found to have a normal platelet adhesiveness, as tested with Hellem's whole blood method

II ACQUIRED CONDITIONS

1 *Haemorrhagic thrombocythaemia*

This is a myeloproliferative disorder characterised by a bleeding tendency despite an increased platelet count The thrombocythaemia may be the only sign, as in primary thrombocythaemia, but is often combined with other myeloproliferative disorders Especially the first patient we investigated case 1, had severe bleeding symptoms, a markedly prolonged Duke bleeding time and a very low degree of adhesiveness as measured with Hellem s whole blood method (66) This patient has since died in a leukaemoid picture Also in three other patients with less marked but obvious bleeding symptoms the Ivy bleeding time was prolonged and the platelet adhesiveness decreased (66) Similar findings have since been reported by McClure et al (158) and Cohen et al (55) Caen et al (44) have reported decreased adhesiveness in many patients with leukaemia

Other investigators have reported a deficiency of platelet factor 3 in thrombocythaemia (104, 158 55) The underlying biochemical abnormality may perhaps not be the same in all patients

2 *Macroglobulinaemia Waldenstrom*

Patients with macroglobulinaemia often exhibit mild bleeding symptoms and a prolonged bleeding time that cannot be explained by a decrease in the platelet count A decreased adhesiveness of platelets has been reported (221 78) In our own investigations the platelet adhesiveness was often decreased in such patients (Appendix II, p 38) The abnormal platelet reactions might be caused by interference of the abnormal globulin with the platelet surface (208) A decrease in factor VIII is often found and may also contribute to the bleeding tendency (177)

3 *Other neoplastic disorders*

While the bleeding tendency in patients with neoplastic diseases is usually caused by thrombocytopenia Borchgrevink et al (26) described a patient with gastric carcinoma and acquired haemorrhagic diathesis with prolonged bleeding time as the only abnormal laboratory finding A similar patient with gastric carcinoma and bleeding tendency with a prolonged bleeding time, despite normal platelet count and coagulation factors and without fibrinolysis and circulating anticoagulants has also been found by Nilsson (177)

4 *Uraemia*

An increased bleeding tendency and a prolonged bleeding time despite a normal platelet count are common in patients with uraemia (137, 145, 261, 229) Various explanations for this combination have been offered Abnormal prothrombin consumption or deficiency

of platelet factor 3 has often been observed (2, 45, 76, 52, 137, 143, 145, 150, 216, 261, 118) Salzman and Neri (229) found a low adhesiveness of platelets with Salzman's method but normal aggregation with ADP Hellem and Odegaard (112) also found a decreased adhesiveness and Castaldi et al (49), a poor ADP aggregation Abnormalities have also been reported by others (213, 200, 211) We have found adhesiveness, as measured with Hellem's whole blood method, to be decreased in many patients with uraemia (Appendix II, p 38) As the platelet adhesiveness in whole blood is dependent on the haematocrit (108), the anaemia of the uraemic patients helps to explain the decrease demonstrated with the whole blood methods On the other hand, several of the uraemic patients had basic diseases that might per se increase the adhesiveness Hellem and Odegaard (112) suggested that the impaired platelet adhesiveness might be explained by the increased amount of urea in the plasma To investigate this possibility volunteers were given 70 g urea by mouth (Appendix III, p 40) It raised the blood urea to 100—170 mg per 100 ml without any effect on platelet adhesiveness

5 *Fibrinolytic conditions*

Dissolution of clots digestion of fibrinogen and other coagulation factors or interference by fibrinolytic split products with blood coagulation (141, 176 174) or platelet function might explain the bleeding tendency in fibrinolytic conditions

In the last few years several reports have appeared on the influence of fibrinolysis and fibrinolytic split products on platelet function (reviewed in 61) Thus Polish authors have reported severe impairment (142, 141, 140), whereas other authors have found moderate effects (130,147) and recently a direct aggregating effect has been reported (5) and Wilson et al (263) found streptokinase to enhance platelet aggregation

We investigated the platelet function after infusion of streptokinase in humans and studied the effect of addition of various agents to platelet-rich plasma *in vitro* (61)

As tested with Hellem's whole blood method, platelet adhesiveness decreased after infusion of streptokinase in man Microscopic examination showed that the platelets adhered less strongly to glass and did not spread out on the glass surface They adhered better and spread on a siliconised surface, however At the same time fibrinogen was decreased and fibrinolytic split products appeared Aggregation with connective tissue suspension was not consistently changed and ADP aggregation was normal The Duke bleeding time was normal or slightly prolonged (3—11 minutes)

Urokinase *in vitro* decreased the adhesion and spreading on a glass slide, but not on a siliconised surface After incubation of platelet rich plasma at 37°C aggregation by connective tissue suspension or by ADP was slightly impaired and spontaneous aggregation was decreased

When studied with the photometric technique, streptokinase in a final concentration of 1600—7000 units per ml plasma aggregated the platelets in citrated platelet-rich plasma after a latency of 2—3 minutes (61), and a sharp drop in the optical density occurred. The dose needed in a given subject was constant but varied from one person to another. Of eleven persons investigated three had platelets that reacted to both 1600/ml and 7000/ml units, four needed the higher concentration, and two responded better to the lower one, whereas in two the platelets failed to aggregate at either concentration. When the supernatant plasma after having been allowed to stand for a short time or after brief centrifugation was transferred to another tube with platelet-rich citrated plasma, the new platelets aggregated within 30 seconds indicating that a release reaction had taken place. The reaction was therefore similar to the effect of adding connective tissue suspension. In vitro experiments with streptokinase were therefore difficult to interpret owing to this direct effect.

Epsilon amino caproic acid (EACA) in vitro counteracted aggregation by connective tissue, ADP or streptokinase.

Purified split products of type D, E or early products of high molecular weight prepared according to Nilehn (172, 173), did not influence the platelet reactions after incubation except that adhesion and spreading on glass was slightly impaired after large doses (2 mg/ml).

To summarise, fibrinolysis produced decreased adhesion of platelets to, and spreading of platelets on, glass and often also moderately decreased aggregation. Similar findings have also been described in afibrinogenaemia (p. 28). In our investigations the fibrinogen was never totally digested and neither lack of fibrinogen nor the appearance of split products could itself explain the findings. Even though factors lost in association with purification might be of importance, an increased amount of fibrinolytic split products interfering with a decreased level of fibrinogen as suggested by Kopec et al. (140) seems most plausible. The bleeding tendency in fibrinolytic conditions can be mediated by different mechanisms and as suggested by these results, impaired platelet function might be a contributory cause.

6. *Scurvy*

In scurvy the patients exhibit an increased bleeding tendency. Cetingil et al. (51) reported impaired platelet aggregation, an observation recently made also by Born and Wright (32) and Wilson et al. (262). The bleeding tendency responds promptly to treatment with ascorbic acid.

7. *Administration of dextran*

Administration of large doses of dextran prolongs the bleeding time in dogs and humans (46, 120, 144, 11, 24, 188) and increased bleeding may be seen (46, 87). In 25 healthy volunteers administration of 1—1.5 g/kg bodyweight of dextran with a mean molecular weight of about 70,000 markedly reduced platelet adhesiveness, as measured with Hel-

lem s whole blood, and plasma-ADP method (69, 70) Factor VIII was decreased and the Ivy bleeding time was prolonged Dextran with a molecular weight of about 40,000 produced but slight changes A decreased platelet adhesion or tendency to aggregation was also found independently by Bygdeman et al (41, 42, 40), Weiss (255) and by Bennett et al (9) The effect did not occur after addition of dextran in corresponding doses *in vitro*, but it was produced *in vivo* The decreased adhesiveness might explain the thrombose-prophylactic effect of dextran (27, 139, 133, 1, 258 and others) Dextran should be avoided in the treatment of bleedings in patients with haemostatic defects

8 *Other drugs*

Clofibrate (Atromid-S©) has been reported to decrease aggregation or adhesiveness of platelets after prolonged administration in vivo (248 47, 48, 91, 217, 89, 94) The changes observed were slight and divergent results were often found when different methods were used O Brien and Heywood (201), Millac et al (163) and Barth and Kommerell (6) found no abnormal adhesiveness to glass

Acetylsalicylic acid has long been known to have an unfavourable effect on patients with bleeding disorders and Beaumont et al (7) found prolongat on of the bleeding time in patients with bleeding disorders but not in normals Blatrix (22) as well as Quick (214) found acetyl-salicylic acid to prolong the bleeding time Morris (166) reported decreased adhesiveness and prolonged bleeding time after oral administration of the drug to normals and abnormal ADP-aggregation of platelets after addition of the drug *in vitro* After administration of acetyl salicylic acid to 10 normals Weiss and Aledort (256) reported impaired platelet aggregation by connective tissue suspension and confirmed the prolongation of the bleeding time Breddin (35) and Evans et al (84) found administration of this drug to reduce the aggregation of platelets We have given acetyl-salicylic acid to 6 normal volunteers without any demonstrable effect on platelet adhesion, as measured by Hellem s whole blood method, or on the Ivy bleeding time (Appendix III p 40) Neither did Hellem (109) find any impaired adhesion with his test It would thus appear that acetylsalicylic acid calls for further investigation for its effect on the aggregation and adhesiveness of platelets

Dipyridamole (Persantin©) has been investigated by Emmons and coworkers (83) who found that it interfered with the formation of platelet aggregates in rabbits *in vivo* In investigations on human blood *in vitro* only small abnormalities were found (82) Rosner et al (220) reported a markedly decreased adhesiveness of platelets We have not been able to confirm this (Appendix III p 40)

Glycerol-guaiacolate has been reported to decrease platelet aggregation with ADP *in vitro* (232) and the platelet adhesiveness after oral administration

(80), but we found normal adhesiveness after oral administration of large doses of the drug (Appendix III, p. 40).

Sulfinpyrazone (Anturan®) and the closely related *phenylbutazone* (Butazolidin®) have been reported to decrease the platelet adhesion and aggregation (237, 170, 169, 209).

Nialamide (Niamidal®) has been reported to decrease platelet aggregation or adhesion (22, 156, 90), but no such effect was found by Eastham (79).

Zweifler (272) found decreased aggregation after treatment of rabbits with large doses or reserpin.

Infusion of polyvinylpyrolidone has been reported to decrease the platelet adhesiveness (230).

In vitro many enzyme inhibitors have been found to impair platelet reactions (236, 105, 168). A suppressive action on various platelet reactions has also been observed by various compounds in vitro, such as certain argenine esters (227), nucleotides (28, 29, 53), guanidino compounds (129), antihistaminics (125, 196, 198, 164, 113), prostaglandin E_1 (138, 81, 35) and others.

The effect of *anticoagulants* on the adhesiveness of platelets has been widely discussed. After administration of dicumarol or related drugs Spooner and Mayer (244) and Wright (265) reported a decreased adhesiveness, as assessed with Wright's method. Many authors found no changes at all (108, 203). We measured the platelet adhesiveness in 20 patients on long term treatment with dicumarol at therapeutic levels (P&P 10—30 %). The mean platelet adhesiveness was normal 32 % (62).

Similarly heparin has been reported by some authors to decrease the adhesiveness of platelets (165, 264, 159), while others have found no effect of conventional doses (194, 225). Our investigations have not revealed any defective adhesiveness in patients treated with heparin (62).

PATHO-PHYSIOLOGICAL REMARKS

Effect of thrombin in primary haemostasis

It has been suggested that thrombin might aggregate platelets by producing a microcoagulation on the platelet surface In a long series of works since 1922 Roskam (219) stressed that several plasma factors are concentrated on the platelet surface, 'l atmosphere plasmatique periplaquettaire', which might yield local reactions that otherwise do not occur Luscher (151) found thrombin to aggregate washed platelets in a protein-free solution Several authors have since confirmed that thrombin aggregates platelets (197, 97, 54 106, 171, 62) The aggregation has been explained by liberation of ADP from platelets by thrombin (136) Johnson et al (131) in electronmicroscopic studies found that fibrin rapidly formed after injury, which suggested that thrombin was formed quicker than usually believed

On the other hand, the normal platelet adhesiveness mostly found in coagulation disorders such as haemophilia (108 224 Appendix I, p 35) or in patients treated with anticoagulants (p 27) argues against thrombin being physiologically important for the adhesion and aggregation of platelets in the early stages of haemostasis This is in agreement with the observation that the primary bleeding time is normal in these disorders It must be remembered, however, that if bleeding recurs, it continues for a long time and the secondary bleeding time is therefore prolonged (25) This evidence is, however, not irrefutable, because even in these conditions thrombin might be produced by extrinsic thromboplastin and thereby possibly explain why the bleeding time is normal The physiological effect of thrombin in the earlier stages of haemostasis is therefore highly controversial, whereas it is clear that in the later stages thrombin is responsible for fibrin formation and initiates viscous metamorphosis and clot retraction and is therefore necessary for producing a firm impermeable clot (268, 269, 151, 152)

Platelet adhesiveness and the von Willebrand bleeding factor

In von Willebrand's disease factor VIII is decreased and another plasma factor (the von Willebrand bleeding factor) is also lacking (182, 183, 180 p 15) The mechanism by which this factor shortens the bleeding time is not known In contrast with what is seen in haemophilic patients, the platelet adhesiveness in von Willebrand's disease is usually decreased when tested with Salzman's method This indicates that the von Willebrand factor might be a cofactor necessary for inducing platelet adhesion or aggregation As the adhesive-

ness increases sharply if the filters are made longer or the rate of flow lower, and as the adhesiveness is normal, as measured with Hellem's method one might wonder whether the factor acts by inducing a reaction that when once started proceeds normally, but is delayed in these patients

Factor VIII and the bleeding factor are both decreased in von Willebrand's disease and seem to vary roughly in parallel Infusion of fresh plasma from a patient with haemophilia A to a patient with von Willebrand's disease is followed by a gradual increase in factor VIII (181) This shows that there must be some connection between the bleeding factor and factor VIII and suggests that the bleeding factor precedes factor VIII A certain amount of von Willebrand factor may be necessary to preserve factor VIII in the plasma Another possible explanation might be that if von Willebrand factor is lacking factor VIII enters another series of reactions and is less available for blood coagulation

More generally speaking the bleeding factor can produce the rise of factor VIII either by stimulating synthesis of this factor or by inhibiting its degradation As both factor VIII and the bleeding factor are present in fraction I—O it is possible that they are bound in a complex

The differentiation between mild and severe cases and the wide fluctuation of the disease is unusual for a dominant hereditary disorder where a defective protein is probably synthesised — in this case a defective unreactive von Willebrand factor In most of such types of genetic abberrations the normal and the defective protein are produced in equal amounts as in mild cases where factor VIII is about 50 % or slightly less By a feed back mechanism the total amount of normal and defective protein i e the normal and the unreactive von Willebrand factor are kept constant but if one of the proteins is more stable the proportion between them will change It is therefore suggested that in severe cases of von Willebrand's disease in active von Willebrand factor is accumulated owing to an intravascular increased consumption of the more vulnerable normal factor

Graham (96) has suggested other models for explaining the many puzzling observations

As for the von Willebrand like disease (Morbus Rita) with normal factor VIII even less is known about the nature of the defective factor The patient benefitted from stored plasma which our patients with von Willebrand's disease did not. The symptoms and laboratory findings suggest that the disorders may be an isolated lack of the von Willebrand factor and a trial in which plasma from von Willebrand patients failed to shorten the bleeding time argues for this hypothesis However the significance of results of this single trial should not be overestimated and the result with stored plasma suggests that the factor must be more stable Other experiments already referred to suggest that the von Willebrand factor is necessary for normal factor VIII level If so the von Willebrand factor should be normal in this patient but another factor may perhaps be activated by it and be responsible for the shortening of the bleeding time might be missing Also other explanations are possible but investigations are difficult owing to lack of appropriate *in vitro* methods

Platelet adhesion and aggregation by connective tissue

Bounameaux (33) *in vitro* and Hugues (123) *in vivo* found that platelets could adhere to connective tissue fibres These investigations were later extended by Hugues *in vivo* (124) and *in vitro* (125) The platelets adhered and aggregated after addition of connective tissue suspension in vitro (270, 197, 121, 243) The aggregation was caused by liberation of ADP from the platelets themselves (121, 243) The aggregation was blocked by EDTA The reactions are probably important for initiating haemostasis

After addition of connective tissue suspension aggregation occurred in samples from all our patients except in those from patients with severe Glanzmann's thrombasthenia In these, however, adhesion took place As already mentioned (p 20) Hirsh et al (114) have described a patient with bleeding symptoms and prolonged bleeding time, besides which the only abnormal finding was a complete absence of platelet aggregation after addition of connective tissue suspension

Platelet adhesion and aggregation by ADP

With Hellem s technique barely any platelets were retained when platelet-rich citrated plasma was passed through the column (108) When red cells were present, retention occurred (108, 110) and Hellem was able to isolate a red cell factor, later identified as adenosine-diphosphate (ADP), that was responsible for this (86) If red cells are first passed through a column of glass beads, platelets in platelet-rich plasma will be retained owing to released ADP (60)

The investigation of Hellem and coworkers prompted extensive experimental work and at present ADP is considered of utmost importance in the early stages of haemostasis (154) The platelets themselves contain large amounts of ADP, which is released after addition of various substances, such as thrombin (p 28), connective tissue suspension (p 29), streptokinase (p 25) and possibly ADP itself (153, 271) and the platelets are then themselves aggregated

Using his optical density method Born (28, 30, 31) studied many factors that influence the aggregation caused by ADP He also studied the inhibitory effect of several nucleotides related to ADP (28, 29, 53) The metabolism of the nucleotides in the platelets has been studied by various authors (228, 242, 117) Anything like a complete review of the investigations on ADP aggregation is beyond the scope of this paper

Platelet adhesiveness and other plasma factors

Platelet reactions probably have much in common with other cell functions such as phagocytosis (73, 93, 167) The adhesion and spreading are probably the result of the platelets trying to engulf an enormous particle Opsonising plasma factors might therefore be of importance Bettex-Galland and Luscher (15, 16) have found immunocomplexes to induce viscous metamorphosis and they discuss the hypothetical influence of the complement system, but the evidence for this is meagre

Brinkhous et al (38) described a platelet aggregating factor TAg Later investigations have shown that this is closely related to, or identical with, fibrinogen (39) Another factor TAg′ (149) is now believed to be thrombin (157) At an intermediate stage of polymerization fibrin was found to aggregate platelets (3, 239) Deykin et al (75) described a heat stable, non-dialysable plasma factor that was consumed in the course of coagulation and increased ADP-induced aggregation The possible effect of fibrinogen has been dis cussed before (pp 22 and 24-25)

Fatty acids of many kinds have also been found to induce platelet aggregation *in vitro* (106, 116) and thrombosis (57, 74) The physiological importance of this effect is obscure Adrenaline, noradrenaline, 5-hydroxytryptamine are other naturally occurring substances capable of clumping platelets (247, 54, 164, 198, 4)

Platelet abnormalities

Morphological abnormalities The underlying abnormality in primary platelet disorders is debatable Evident abnormalities are present in the platelets in the disorder of Bernard and Soulier (13, p 21) In Glanzmann's thrombasthenia electronmicroscopy has revealed abnormalities but no specific changes (155, 43, 148)

Biochemical abnormalities In patients with Glanzmann's severe thrombasthenia the platelets have been reported to contain a decreased amount of fibrinogen (127, 267, 43) or of ATP and glucolytic enzymes (98, 155) In a survey of the French cases Caen et al (43) found the decrease in fibrinogen to be variable and concluded that it is difficult to believe that this anomaly was responsible for the failure of aggregation They believed that defects in platelet adhesion and clot retraction are related to deficient adsorption properties of the platelet membrane rather than due to a metabolic derangement

After addition of connective tissue suspension aggregation occurred in samples from all our patients except in those from patients with severe Glanzmann's thrombasthenia. In these, however, adhesion took place. As already mentioned (p. 20) Hirsh et al. (114) have described a patient with bleeding symptoms and prolonged bleeding time, besides which the only abnormal finding was a complete absence of platelet aggregation after addition of connective tissue suspension.

Platelet adhesion and aggregation by ADP

With Hellem's technique barely any platelets were retained when platelet-rich citrated plasma was passed through the column (108). When red cells were present retention occurred (108, 110) and Hellem was able to isolate a red cell factor, later identified as adenosine diphosphate (ADP), that was responsible for this (86). If red cells are first passed through a column of glass beads, platelets in platelet-rich plasma will be retained owing to released ADP (60).

The investigation of Hellem and coworkers prompted extensive experimental work and at present ADP is considered of utmost importance in the early stages of haemostasis (154). The platelets themselves contain large amounts of ADP which is released after addition of various substances, such as thrombin (p. 28), connective tissue suspension (p. 29), streptokinase (p. 25) and possibly ADP itself (153, 271) and the platelets are then themselves aggregated.

Using his optical density method Born (28, 30, 31) studied many factors that influence the aggregation caused by ADP. He also studied the inhibitory effect of several nucleotides related to ADP (28, 29, 53). The metabolism of the nucleotides in the platelets has been studied by various authors (228, 242, 117). Anything like a complete review of the investigations on ADP aggregation is beyond the scope of this paper.

Platelet adhesiveness and other plasma factors

Platelet reactions probably have much in common with other cell functions such as phagocytosis (73, 93, 167). The adhesion and spreading are probably the result of the platelets trying to engulf an enormous particle. Opsonising plasma factors might therefore be of importance. Bettex-Galland and Luscher (15, 16) have found immunocomplexes to induce viscous metamorphosis and they discuss the hypothetical influence of the complement system, but the evidence for this is meagre.

Brinkhous et al. (38) described a platelet aggregating factor TAg. Later investigations have shown that this is closely related to, or identical with, fibrinogen (39). Another factor TAg' (149) is now believed to be thrombin (157). At an intermediate stage of polymerization fibrin was found to aggregate platelets (3, 239). Deykin et al. (75) described a heat stable, non-dialysable plasma factor that was consumed in the course of coagulation and increased ADP-induced aggregation. The possible effect of fibrinogen has been discussed before (pp. 22 and 24-25).

Fatty acids of many kinds have also been found to induce platelet aggregation *in vitro* (106, 116) and thrombosis (57, 74) The physiological importance of this effect is obscure Adrenaline, noradrenaline, 5-hydroxytryptamine are other naturally occurring substances capable of clumping platelets (247, 54, 164, 198, 4)

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HOW TO DIAGNOSE A PRIMARY HAEMORRHAGIC DEFECT

In a patient with a history of bleeding suggesting a primary platelet defect the bleeding time should be measured The Duke bleeding time will be prolonged only in severe cases, but the Ivy bleeding time is much more sensitive and

Table I Survey of *haemorrhagic disorders with prolonged bleeding time**

	Bleeding time		Coagulation time	Prothrombin consumption	Platelet adhe[illegible] according to	
	Duke	Ivy			Hellem	S[illegible]
von Willebrand's disease severe form	prolonged	prolonged	prolonged or normal	abnormal	normal	lo
mild form	often normal	prolonged	normal	often abnormal	normal	lo
Morbus Rita **	prolonged	prolonged	normal	normal	normal	l[illegible]
Glanzmann's severe thrombasthenia	prolonged	prolonged	normal	often normal	absent	al
Moderately severe thrombasthenia	often normal	prolonged	normal	often normal	mostly low	m[illegible] lo
Mild thrombasthenia	often normal	prolonged	normal	mostly normal	mostly low	o[illegible] lo
Distr thromb hemorragip Bernard & Soulier)	prolonged	prolonged	normal			
Thrombocytopathy	often prolonged	prolonged	often prolonged	abnormal	?	?
Afibrinogenaemia	prolonged	prolonged	—	—	low	lo
Thrombocytopenia	often prolonged	prolonged	normal or prolonged	abnormal	variable	va
Haemophilia A	normal***	normal	prolonged	abnormal	normal	n[illegible]
[illegible] coagulation [illegible]	normal***	normal	prolonged	abnormal	normal	n[illegible]

* Owing to biological variation or technical errors in individual cases the results especially single determinations may deviate from the general trend

** Similar conditions with a decrease of various coagulation factors have been reported

*** Primary bleeding time is normal but secondary bleeding time may be prolonged

APPENDIX I

PLATELET ADHESIVENESS IN PATIENTS WITH COAGULATION DISORDERS

To evaluate the influence of coagulation factors on the platelet adhesiveness various experiments were performed. In haemophilia Wright (266) reported a decreased adhesiveness with her method whereas Hellem (108) and Salzman (224) found normal adhesiveness. A decreased adhesiveness in von Willebrand's disease, as tested with Salzman's method, has been reported by several authors (224, 246, 161). Recently O'Brien and Heywood (202) found impaired platelet adhesiveness in von Willebrand's disease when native or heparinised blood was passed through columns at a high, but not at a low, rate. When tested with Hellem's method platelet adhesiveness in von Willebrand's disease had earlier been found to be normal (67), but these investigations were now extended to evaluate the platelet adhesiveness, as tested with the method of Salzman (224).

CLINICAL MATERIAL

Patients with various kinds of haemophilia or von Willebrand's disease were investigated. The patients with haemophilia A and B have earlier been described by Ramgren et al. (215). The von Willebrand patients were mainly the same ones as were studied in an earlier investigation and were characterised by dominant heredity, prolonged bleeding time and decreased factor VIII. Besides five patients with haemophilia C (PTA deficiency, f XI-deficiency) and one patient with complete factor VII deficiency. The cases of PTA deficiency were diagnosed by a modified TGT-test (177), and the case of factor VII-deficiency by abnormal P&P-test, normal Stypven time and inability to correct abnormality in other patients with deficiency of this factor.

METHODS

Platelet adhesiveness was measured according to Hellem's methods for whole blood as described before (p. 9). In a modification of the method no citrate or other anticoagulants were added and the blood was allowed to stand for various intervals before it was passed through the column. After the passage the blood was often collected in fractions of 1 ml.

Salzman's method was used according to the original method (p. 11, 224, 60).

RESULTS

The results are given i Tables 2 and 3.

Experiments with Hellem's method

The platelet adhesiveness in citrated whole blood was normal in the various coagulation disorders.

the use of Salzman s method for testing platelet adhesiveness and in doubtful cases the response to administration of fraction I—O can clinch the diagnosis Differentiation between plasma defects and primary platelet defects is clinically important A decreased factor VIII indicates a plasma defect but, as mentioned above (p 17) there exists a bleeding disorder of similar type due to a defect of a plasma factor, but with a normal factor VIII Glanzmann s severe thrombasthenia is characterised by a defective clot retraction and absence of platelet aggregation Salzman s method shows low adhesion in all these conditions, while a normal adhesion with Hellem s method is more compatible with von Willebrand s disease The type of condition that we have called moderately severe thrombasthenia shows so many specific signs, such as absence of spontaneous aggregation in platelet-rich plasma and the peculiar swelling of the platelets when suspended in citrate, that it can be diagnosed with certainty by a trained investigator Most difficult to diagnose are the cases we call mild thrombasthenia because the only laboratory findings are a prolonged Ivy bleeding time and a more or less markedly decreased adhesiveness, as measured with Hellem's and Salzman s methods, and in these patients all other coagulation disorders must be excluded and the condition is also often difficult to differentiate from normality As the bleeding symptoms are usually mild and no specific treatment is available, the diagnosis in these patients is not so important as in those with plasma defects

To conclude, severe bleeding disorders can often be easily and firmly diagnosed with simple qualitative tests For most mild conditions there is no single test that can decide the diagnosis, which must instead be made on the basis of laboratory findings and clinical signs and symptoms, of the history of the disease and familial investigation The laboratory findings often vary from time to time owing to biological fluctuation of the disease and experimental errors In mild cases it is therefore not recommended to make a diagnosis on the basis of a single investigation, repeated examinations often being necessary to diminish methodological errors

When the blood was passed through Hellem's column immediately after withdrawal without addition of anticoagulants the platelet adhesiveness was the same in various kinds of haemophilia, von Willebrand's disease and normals, and was about 50 %, which was higher than in citrated blood. When allowed to stand the platelet adhesiveness decreased in haemophilic blood and tended to become lower than in citrated blood. The same increase in the number of adhering platelets with every fraction, as found earlier in citrated blood (60), was found also in the not anticoagulated blood.

Experiments with Salzman's method

The investigations revealed that in patients with von Willebrand's disease the platelet adhesiveness was usually decreased, as tested with Salzman's method, whereas the adhesiveness was seldom low in haemophilic patients or normals (Table 3).

The results of the investigations on von Willebrand patients are discussed on p. 16 and on haemophilic patients on p. 28.

Table 2 *Platelet adhesiveness in coagulation disorders*
Platelet adhesiveness (per cent) as tested with Hellem s technique but with the modification that anticoagulants in some tests were omitted

	Whole blood without anticoagulants			Citrated whole blood	Number of individuals investigated
Time between withdrawal of blood and testing	5 min	30 min	1 1/2 hours	1 hour	
Coagulation disorder					
Severe haemophilia A (Factor VIII deficiency)	50	27	21	28	10
Severe or mild haemo philia B (Factor IX deficiency)	53	32	29	34	3
Haemophilia C PTA (Factor XI deficiency)	49	—	—	30	5
Factor VII deficiency	47	—	—	25	1
von Willebrand s disease	57	—	—	26	10
Normal	50	—	—	31	10

Table 3 *Platelet adhesiveness in von Willebrand s disease as tested with Salzman s method*

	von Willebrand	Haemophilic	Normal
Total number	67	12	86
Number of patients with low adhesiveness (< 21 %)	51	2	11
Number of patients with high adhesiveness (> 20 %)	16	10	75
Percentage with low adhesiveness (< 21 %)	76	17	13

Table 4 *Platelet adhesiveness in patients with macroglobulinaemia*

Initials	Born	Macroglob g/100 ml	Haemoglob g/100 ml	Bleeding Duke min	time Ivy min	Platelet count per mm³	Platelet adhesiveness (%) accord. to Hellem: whole blood	plasma + ADP 0 05	0 10 µg/ml	Factor VIII %	Fibrinogen g/1[00] ml
V Z	1896	6 5	8 3	4		104 000	15	4	8	28	0 16
A R	1894	5 2	8 3	2		170 000	24	17	43	58	0 28
S S	1899	1 1	12 5	2		360 000	24	37	62	48	0 28
L R	1914	2 4	12 5	2		126 000	27	16		58	0 36
M O	1918	2 5	8 9	7	> 30	95 000	28			52	0 29
M W	1886	1 8	7 6	2	> 30	372 000	30			52	0 65

Table 5 *Platelet adhesiveness in patients with uraemia*

Initials	Sex	Born	Urea mg/100 ml	Creatinine mg/100 ml	Haemoglobin g/100 ml	Platelet count per mm³	Platelet adhesiveness of Hellem (%): whole blood	plasma + ADP 0 05 µg/ml	0 10 µg/ml
N A	M	1892	340	15	9 2	344 000	22		
S A	F	1897	200	6	5 8	241 000	13	2	33
M J	M	1900	125	5	9 1	324 000	31	11	44
S L	F	1902	355	14	5 8	219 000	23		
E A	M	1916	280	10	7 4	79 000	19	22	38
G J	M	1902	138	6	9 6	378 000	28	19	45
G K	M	1914	270	10	8 3	156 000	32	8	22
R P	M	1932	116	5	9 4	196 000	29	19	32
M M	F	1903	207	9	5 0	141 000	21	10	39
G H	M	1921	100	7	6 4	285 000	[illegible]	16	27
J S	M	1901	520	29	6 9	170 000	16	3	13
M B	F	1902	228	14	8 0	198 000	36	21	31
B N	M	1917	275	12	6 6	410 000	20	13	50
K J	F	1906	112	5	8 3	240 000	33	30	54
n = 14		Mean	233	10 5	7 6	241 000	25	14 5	36

APPENDIX III

PLATELET ADHESIVENESS AFTER ADMINISTRATION OF VARIOUS DRUGS

Acetyl salicylic acid, dipyridamole (Persantin®), glyceryl-guaiacolate, or urea have been associated with decreased platelet adhesiveness (for references, see pp 26—27). This study reports our own preliminary results on the effect on platelet adhesiveness of these drugs after administration to volunteers.

Table 6 *Platelet adhesiveness after ingestion of urea*
Investigation 1 hour after ingestion of 1 g/kg bodyweight of urea in 8 normals

	Bleeding time Ivy min	Urea mg/100 ml	Platelet adhesiveness (%) according to Hellem in whole blood	plasma + ADP 0.05 µg/ml	plasma + ADP 0.10 µg/ml
Before	11		36	28	56
After	11	131	37	25	51

Table 7 *Effect of i.v. infusion of Dipyridamole (Persantin®) on platelet adhesiveness*
150 mg Persantin in 500 ml 0.9 % sodium chloride was infused within 1 hour to 5 normals.

	Platelet adhesiveness in % according to Hellem's methods: whole blood	plasma + ADP 0.05 µg/ml	plasma + ADP 0.10 µg/ml	Bleeding time Ivy (min.)
Before	31	24	40	10'30"
After	29	16	36	12'48"

Table 8 *Effect of dipyridamole (Persantin®) on platelet adhesiveness after addition in vitro*
0.5 mg dipyridamole was either present in the tube together with 1 ml citrate at collection of 9 ml blood or added to 10 ml citrated blood or 5 ml platelet rich plasma just before passage through the column. The platelet adhesiveness was tested according to Hellem's methods and experiments were performed 6 times in blood from different normals

	Platelet adhesiveness in %: whole blood	plasma + ADP 0.05 µg/ml	plasma + ADP 0.10 µg/ml
Control (TRIS buffer)	32	11	43
Added at withdrawal	33	15	49
Added just before passage	32	16	43

MATERIAL

The drugs were administered to healthy volunteers, preferably those with adhesiveness in the upper part of the normal range, in whom a suppressive action might more easily be detected The volunteers were mostly male military medical orderlies about 20 years of age The mode of administration and the doses given are reported in Tables 6, 7, 9 and 10 In one series the drug was added in vitro immediately after withdrawal of the blood or just before it was passed through the column (Table 8)

METHODS

Platelet adhesiveness was tested according to Hellem's methods for whole blood and plasma-ADP (67, p 9) The bleeding time was determined according to Ivy as described on p 7

RESULTS

None of the drugs produced any marked change in the platelet adhesiveness or the bleeding time in these investigations (Tables 6—10) When a drop of platelet-rich plasma was placed on a glass slide under a cover slip, the platelets aggregated and adhered to the glass in a normal way

The results are discussed on pp 24 and 26—27

Table 9 *Effect of administration of acetyl salicylic acid on platelet adhesiveness*

Acetyl salicylic acid in a dose of 1 g four times a day was given for 24 hours to 6 normals The last dose was given 1 1/2 hours before blood was drawn for testing Platelet adhesiveness was determined according Hellem's whole blood method

	Platelet adhesiveness %	Bleeding time Ivy
Before	37	8 20
After	41	8 55

Table 10 *Effect of administration of glyceryl-guaiacolate on platelet adhesiveness*

Glyceryl guiacolate 100 mg 4 times a day was orally administered for 24 hours to 7 volunteers. The last dose was taken 1 1/2 hours before withdrawal of blood for testing Platelet adhesiveness was investigated by Hellem's method.

	Platelet adhesiveness %	Bleeding time Ivy
Before	36	9
After	40	9

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REFERENCES

1 *Ahlberg A Nylander G Robertson B, Cronberg S & Nilsson I M* Dextran in prophylaxis of thrombosis in fractures of the hip Acta chir scand Suppl in press

2 *Altschuler G Marcus A J & Ullman H C* Platelets and platelet phosphatides in uremia Blood *16* 1439 1960

3 *Apitz K* Über Profibrin V Agglutination von Blutplättchen durch Profibrin Z ges exp Med *105* 89 1939

4 *Ardlie N G Glew G & Schwartz C J* Influence of catecholamines on nucleotide induced platelet aggregation Nature Lond *212* 415 1966

5 *Barnhart M I Cress D C Henry R L & Riddle J M* Influence of fibrinogen split products on platelets Thrombos Diathes haemorrh (Stuttg) *17* 78 1967

6 *Barth P & Kommerell B* Wirkung von Clofibrat auf die Blutgerinnung Med Klin *61* 1466 1966

7 *Beaumont J L Caen J & Bernard J* Influence de l acide acetyl salicylique dans les maladies hemorragiques Sang *27* 243 1956

8 *Bennett E & Dormandy K* Pool s cryo precipitate and exhausted plasma in the treatment of von Willebrand s disease and factor XI deficiency Lancet 2 731 1966

9 *Bennet P N Dhall D P McKenzie F N & Matheson N A* Effects of dextran infusion on the adhesiveness of human blood platelets Lancet 2 1001 1966

10 *Benitegeat J Verger P Moulinier J Mesnier F Beauxieux Y J Bosseau M & de Joigny C* Un cas d afibrinogene mie congenitale Proc 10th Congr europ Soc Haemat Strasbourg 1965 Part II Karger Basel/New York 1967 p 977

11 *Bergentz S E Esken O & Nilsson I M* The effect of dextran of various molecular weight on the coagulation in dogs Thrombos Diathes haemorrh (Stuttg) *6* 15 1961

12 *Bernard J Caen J & Maroteau P* La dystrophie thrombocytaire hemorragipare congenitale Rev Hemat *12* 222 1957

13 *Bernard J & Soulier J P* Sur une nouvelle variete de dystrophie thrombocytaire hemorragipare congenitale Sem Hop Paris *24* 3217 1948

14 *Bettex Galland M & Luscher E F* Studies on the metabolism of human blood platelets in relation to clot retraction Thrombos Diathes haemorrh. (Stuttg) *4* 178 1960

15 *Bettex Galland M & Luscher E F* Untersuchungen uber die Auslosung der viskosen Metamorphose der menschlichen Blutplattchen durch Immunokomplexe Path. Microbiol (Basel) *27* 533, 1964

16 *Bettex Galland M Luscher E F Simon G & Vassalli P* Induction of viscous metamorphosis in human blood platelets by means other than by thrombin Nature (Lond) *200* 1109 1963

17 *Biggs R & MacFarlane R G* Human blood coagulation and its disorders Oxford Blackwell 1962

18 *Bjorkman S E* A new method for enumeration of platelets Acta haemat, 22 377 *1959*

19 *Bjorlin G & Nilsson I M* Oral surgery in patients with coagulopathies Acta odont scand *21* 99 1963

20 *Bjorlin G & Nilsson I M* Oral surgery in patients with coagulopathies II Odontologisk Revy (Stockh) *16* 216 1965

21 *Blackburn E K Monaghan J H Lederer H & Macfie J M* Christmas disease associated with primary capillary abnormalities Brit med J *1* 154 1962

22 *Blatrix C* Allongement du temps de saig-

nement sous l influence de certains medicaments Nouv Rev Franc Hemat 3 346 1963

23 *Blomback, B & Blomback M* Purification of human and bovine fibrinogen Arkiv Kemi *10* 415 1956

24 *Bloom, W L Fowler N O Ward J A & French R H* Hemodilution and changes in hemostasis induced by dextran An experiment in dogs with and without plasma volume expansion J surg Res *3* 152 1963

25 *Borchgrevink C F & Waaler B A* The secondary bleeding time A new method for the differentiation of hemorrhagic diseases Acta med scand *162* 361 1958

26 *Borchgrevink C F Hellem A Voss D & Waaler B A* Acquired hemorrhagic diathesis of unknown nature Thrombos Diathes haemorrh (Stuttg) 3 187 1959

27 *Borgstrom S Gelin L E & Zederfeldt B* The formation of vein thrombi following tissue injury an experimental study in rabbits Acta chir scand Suppl 247 1959

28 *Born G V R* Aggregation of blood platelets by adenosine diphosphate and its reversal Nature (Lond) *194* 927 1962

29 *Born G V R* Strong inhibition by 2 chloroadenosine of the aggregation of blood platelets by adenosine diphosphate Nature (Lond) *202* 95 1964

30 *Born G V R* Effects of adenosine diphosphate (ADP) and related substances on the adhesiveness of platelets in vitro and in vivo Brit J Haemat *12* 37 1966

31 *Born G V R & Cross M J* The aggregation of blood platelets J Physiol *168* 178 1963

32 *Born G V R & Wright H I* Platelet adhesiveness in experimental scurvy Lancet *I* 477 1967

33 *Bounameaux Y* L accolement des plaquettes aux fibres sous endotheliales Compt rend Soc Biol (Paris) *153* 865 1959

34 *Brecher C & Cronkite E P* Morphology and enumeration of human blood platelets J appl Physiol 3 365 1950

35 *Breddin K* Personal communication

36 *Breddin, K & Bauke J* Thrombozyten agglutination und Gefasskrankheiten Blut *11* 144 1965

37 *Breddin K & Burck K H* Zur Klinik der Thrombozytenfunktionsstorungen unter besonderer Berucksichtigung der Ausbreitungsfahigkeit der Thrombozyten an silikonisierten Glasflachen Thrombos Diathes haemorrh (Stuttg) *9* 525, 1963

38 *Brinkhous K M LeRoy E C Cornell W P Brown, R C Hazlehurst J L & Vennart G P* Macroscopic studies of platelet agglutination nature of thrombocyte agglutinating activity of plasma Proc Soc exp Biol Med (N Y) *98* 379 1958

39 *Brinkhous K M Read M S & Mason R G* Plasma thrombocyte agglutinating activity and fibrinogen Synergism with adenosine diphosphate Lab Invest, *14* 335 1965

40 *Bygdeman E & Eliasson R* Effect of dextrans on platelet adhesiveness and aggregation Scand J clin Lab Invest *20* 17 1967

41 *Bygdeman S Eliasson R & Gullbring B* Dextrans inverkan på trombocyternas aggregationsegenskaper Experimentell studie Medicinska Riksstamman Stockholm 1965

42 *Bygdeman S Eliasson R & Gullbring B* Effect of dextran infusion on the adenosine diphosphate induced adhesiveness and the spreading capacity of human blood platelets Thrombos Diathes haemorrh (Stuttg) *15* 451 1966

43 *Caen J P Castaldi P A Leclerc J C Inceman S Larrieu M J Probst M & Bernard J* Congenital bleeding disorders with long bleeding time and normal platelet count I Glanzmann s thrombasthenia (Report of fifteen patients) Amer J Med *41* 4 1966

44 *Caen J Sinakos Z. Sultan, Y Vainer, H & Bernard J* Les troubles du fonctionnement des plaquettes dans les leucemies myeloides chroniques Nouv Rev Franç Hemat *6* 719 1966

45 *Cahalane S F, Johnson S A Monto, R W & Caldwell M J* Acquired thrombo

cytopathy observations on the coagulation defect in uremia Amer J clin Path 30 507 1958

46 *Carlone J V Furth F W Scott Jr R & Crosby W H* An haemostatic defect associated with dextran infusion Proc Soc exp Biol (N Y) *85* 101 1954

47 *Carson P McDonald L Pickard S Pilkington T Davies B & Love F* Effect of atromid on platelet stickiness J atheroscler Res 3 619 1963

48 *Carson P McDonald L Pickard S Pilkington T Davies B & Love F* Effects of clofibrate with androsterone (Atromid) and without androsterone (Atromid S) on blood platelets and lipids in ischaemic heart disease Brit. Heart. J *28* 400 1966

49 *Castaldi P A Larrieu M J & Caen J* Availability of platelet factor 3 and activation of factor XII in thrombasthenia. Nature (Lond) *207* 422 1965

50 *Castaldi P A Rozenberg M C & Stewart J H* The bleeding disorder in uraemia A qualitative platelet defect Lancet II 66 1966

51 *Cetangil A I Ulutin O N & Karaca M* A platelet defect in a case of scurvy Brit. J Haemat. *4* 350 1958

52 *Cheney K & Bonnin J A* Haemorrhage platelet dysfunction and other coagulation defects in uremia. Brit. J Haemat. *8* 215 1962

53 *Clayton S Born G V R. & Cross M J* Inhibition of the aggregation of blood platelets by nucleosides Nature (Lond) *200* 138 1963

54 *Clayton S & Cross M J* The aggregation of blood platelets by catecholamines and by thrombin J Physiol. *169* 82P 1963

55 *Cohen I Djaldetti M Karp M & Vries A* Thrombocytosis with impaired platelet adhesiveness and platelet factor 3 availability New Istanbul Contrib clin Sci 9 20 1966

56 *Combrisson A Le Bolloch A G Debray J & Benhamou J P* Deficit en facteur antihemophilique B associe a un allongement du temps de saignement. Sang *28* 137 1957

57 *Connor W E. Hoak J C & Warner E D* Massive thrombosis produced by fatty acid infusion J clin Invest. *42* 860 1963

58 *Cornu P Larrieu M J Caen J & Bernard J* Donnees nouvelles concernant la maladie de Willebrand (angiohemophilie) Rev franç Et. clin biol 5 614 1960

59 *van Creveld S & Mochtar I A* Von Willebrand s disease — a plasma deficiency cause of the prolonged bleeding time Ann Paediat 194 37 1960

60 *Cronberg S* Methodological problems in testing of platelet adhesiveness. Scand. J Haemat *4* 385 1967

61 *Cronberg S* Effect of fibrinolysis on adhesion and aggregation of human platelets Thrombos Diathes. haemorrh. (Stuttg) in press

62 *Cronberg S* unpublished results

63 *Cronberg S. & Nilsson I M* Coagulation studies after administration of a fat emulsion Intralipid® Thrombos. Diathes haemorrh (Stuttg) *18* 664 1967

64 *Cronberg S & Nilsson I M* Investigations of patients with mild thrombasthenia — a haemorrhagic disord r with prolonged bleeding time probably due to a primary platelet defect. Acta med scand in press.

65 *Cronberg S & Nilsson I M* Investigations in a family with thrombasthenia of moderately severe type with 16 affected members Scand J Haemat. *5* 17 1968

66 *Cronberg S Nilsson I M & Gydell K* Haemorrhagic thrombocythaemia due to defective platelet adhesiveness. Scand J Haemat. 2 208 1965

67 *Cronberg S. Nilsson I M & Silwer J* Studies on platelet adhesiveness in von Willebrand s disease Acta med scand *180* 43 1966

68 *Cronberg S Nilsson I M & Zetterqvist E.* Investigation of a family with members with both severe and mild degree of thrombasthenia. Acta paediat. scand. *56* 189 1967

69 *Cronberg S Robertson B & Nilsson I M* Sankt trombocytadhesivitet efter dextrantillforsel. Medicinska Riksstamman Stockholm 1965

70 *Cronberg S Robertson B Nilsson I M & Nilehn J E* Suppressive effect of dextran on platelet adhesiveness Thrombos Diathes haemorrh (Stuttg) *16* 384, 1966

71 *Cross M J* Effect of fibrinogen on the aggregation of platelets by adenosine di phosphate Thrombos Diathes haemorrh (Stuttg) *12* 524 1964

72 *Cullum C Cooney D P & Schier S L* Familial thrombocytopenic thrombocyto pathy Brit J Haemat *13* 147 1967

73 *David Ferreira J F* Sur la structure et le pouvoir phagocytaire des plaquettes sangui nes Z Zellforsch *55* 89 1961

74 *Day, H J Feuell W & Soloff L A* Thrombosis in the dog produced by single rapid infusion of long chain fatty acids Amer J med Sci *253* 83 1967

75 *Deykin D Pritzker G R. & Scolnick F M* Plasma co factors in adenosine di phosphate induced aggregation of human platelets Nature (Lond) *208* 296 1965

76 *Donner, L & Neuwirtova R* Hemostatic defect of acute and chronic uremia Thrombos Diathes haemorrh (Stuttg) *5* 319 1960

77 *Durmandy A Hardisty R M & Mac Pherson J* Christmas disease and capillary abnormality Brit med J *I* 566 1962

78 *Doumenc J Prost J Samama M & Boutier J* Anomalie de l agregation au cours de la maladie de Waldenstrom (A propos de 3 cas) Nouv Rev Franç He mat 6 734 1966

79 *Eastham R D* Attempted reduction of adhesive platelets after cardiac infarction Lancet 2 541 1964

80 *Eastham R D & Griffiths E P* Reduc tion of platelet adhesiveness and prolonga tion of coagulation time of activated plas ma by glycerol guajacolate Lancet *I* 795 1966

81 *Emmons P R Hampton J R Harrison M J G Honour A J & Mitchell J R A* Effect of Prostaglandin E on platelet behaviour in vitro and in vivo Brit med J *2* 468, 1967

82 *Emmons P R. Harrison, M J G Honour, A J & Mitchell J R A* Effect of di pyridamole on human platelet behaviour Lancet *2* 603 1965

83 *Emmons P R Harrison M J G Honour, A J & Mitchell, J R A* Effect of a pyrimido pyrimidine derivate on thrombus formation in the rabbit Nature (Lond) *208* 255 1965

84 *Evans G Nishizawa, E E Packham, M A & Mustard, J F* The effect of acetyl salicylic acid (aspirin) on platelet func tion Abstr 3rd Conf on Platelets Oak Ridge Blood *30* 550 1967

85 *Friedman L. L. Bowie F J W Thomp son Jr J H, Brown A L & Owen Jr C A* Familial Glanzmann s thrombasthe nia Mayo Clinic Proc *39* 908 1964

86 *Gaarder A Jenson J, Laland S Hellem, A & Owren P A* Adenosine diphospha te in red cells as a factor in the adhesive ness of human blood platelets Nature (Lond) *192* 531 1961

87 *Garzon, A A Fink L H Shaftan G W & Karlson K E* Blood loss associated with administration of low molecular weight dextran J thorac cardiov Surg *48* 873 1964

88 *Gaston L W Edson J R & Hartmann J R* Factor IX deficiency and a prolonged bleeding time New Engl J Med *275* 252 1966

89 *Gibelli A Frandoli G Giarola P & de Nicola P* Fibrinolytic activity and reduc tion of platelet adhesiveness following pro longed administration of ethyl chlorophe noxy isobutyrate (Atromid S) Hemostase 6 285 1966

90 *Gibinski K Waledowska J & Zagornik J* Blood platelet adhesiveness and the pro tective action of nialamid in atherosclero tic dysbasia J atheroscler Res *4* 461 1964

91 *Gilbert J B & Mustard J F* Some ef fects of Atromid on platelet economy and blood coagulation in man J atheroscler Res *3* 623 1963

92 *Glanzmann E* Hereditare hamorrhagische Thrombasthenie Ein Beitrag zur Pathologie

der Blutplattchen. Jahrb . Kinderheilk 88 1 1918

93 *Glynn M F Movat H Z Murphy E A & Mustard J F* Study of platelet adhesiveness and aggregation with latex particles J Lab clin Med 65 179 1965

94 *Glynn M F Murphy E A & Mustard J F* Effect of clofibrate on platelet economy in man. Lancet I 447 1967

95 *Gobbi F Antonucci M & Ascari E.* Angioemofilia B Haemat lat. 3 363 1960

96 *Graham J B* The genetics of hemophilia Series haemat (Copenhagen) 7 14 1965

97 *Grette K* Studies on the mechanism of thrombincatalyz d hemostatic reactions in blood platelets Acta physiol scand Suppl 195 1962

98 *Gross R Gerok W Lohr G W Vogell W Waller H D & Theopold W* Uber die Natur der Thrombasthenie Thrombopathie Glanzmann Naegeli. Klin Wschr 38 193 1960

99 *Grottum* Personal communication

100 *Gugler E* Angiohamophilie Schweiz med Wschr 90 534 1960

101 *Gugler E & Luscher E F* Platelet function in congenital afibrinogenemia Thrombos Diathes haemorrh (Stuttg) 14 361 1965

102 *Hardisty R M Dormandy K M & Hutton R A.* Thrombasthenia Studies on three cases Brit J haemat 10 371 1964

103 *Hardisty R. M & Hutton R A* Bleeding tendency associated with new abnormality of platelet behaviour Lancet 1 983 1967

104 *Hardisty R M & Wolff H H* Haemorrhagic thrombocythaemia a clinical and laboratory study Brit J Haemat 1 390 1955

105 *Harrison M J G Emmons P R & Mitchell J R A* The effect of sulphhydryl and enzyme inhibitors on platelet aggregation in vitro Thrombos Diathes haemorrh (Stuttg) 16 122 1966

106 *Haslam R. J* Role of adenosine diphosphate in the aggregation of human blood platelets by thrombin and by fatty acids Nature (Lond) 202 765 1964

107 *Hegglin, R Gross R & Lohr G W* Anomalie Hegglin May (Polyphyle Reifungsstorung) Schweiz. med Wschr 94 1357 1964

108 *Hellem A J* The adhesiveness of human blood platelets in vitro Scand J clin Lab Invest. Suppl 51 1960

109 *Hellem A J* Personal communication

110 *Hellem A J Borchgrevink C F & Ames S B* The role of red cells in haemostasis the relation between haematocrit bleeding time and platelet adhesiveness Brit. J Haemat 7 42 1961

111 *Hellem A J Odegaard A E & Skalbegg B A* Investigations on adenosine diphosphate (ADP) induced platelet adhesiveness in vitro Part 1 The ADP platelet reaction in various experimental conditions Thrombos Diathes haemorrh (Stuttg) 10 61 1963

112 *Hellem A J Odegaard A E. & Skalbegg B A* Platelet adhesiveness in chronic renal failure Xth Congr Internat Soc Haematol Stockholm 1964

113 *Herrman R G & Frank J* Effect of adenosine derivatives and antihistaminics on platelet aggregation Proc Soc exp Biol (N Y) 123 654 1966

114 *Hirsh J Castelan D J & Loder P B* Spontaneous bruising associated with a defect in the interaction of platelets with connective tissue Lancet 2 18 1967

115 *Hirsh J Castelan D J & Loder P B* Discussion to Platelets — a Portsmouth syndrome Lancet 2 469 1967

116 *Hoak J C Warner E. D & Connor W E.* Platelets fatty acids and thrombosis Circ Res 20 11 1967

117 *Holmsen H* Adenine nucleotide metabolism in platelet and plasma In Kowalski E. and Niewiarowski S (eds) Biochemistry of blood platelets Academic Press London and New York 1967 p 81

118 *Horowitz H I Cohen B D Martinez P & Papayoanou M F* Defective ADP induced platelet factor 3 activation in uremia Blood 30 331 1967

119 *Horowitz H I & O'Leary D* Von Willebrand's disease a critical evaluation of diagnostic criteria N Y State J Med *65* 2236 1965

120 *Howard J M Teng C T & Loeffler R K.* Studies of dextrans of various molecular sizes Ann Surg *143* 369 1956

121 *Hovig T* Release of a platelet aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline extract of tendons Thrombos Diathes haemorrh (Stuttg) *9* 264 1963

122 *Hugues J* Agglutination precoce des plaquettes au cours de la formation du clou hemostatique Thrombos Diathes haemorrh (Stuttg) *3* 177, 1959

123 *Hugues J* Accolement des plaquettes au collagene Compt. rend Soc Biol (Paris) *154* 866 1960

124 *Hugues J* Accolement des plaquettes aux structures conjonctives perivasculaires Thrombos Diathes haemorrh (Stuttg) *8* 241 1962

125 *Hugues J & Lapiere C M* Nouvelles recherches sur l'accolement des plaquettes aux fibres de collagene Thrombos Diathes haemorrh (Stuttg) *11* 327 1964

126 *Inceman S Caen J & Bernard J* Aggregation, adhesion and viscous metamorphosis of platelets in congenital fibrinogen deficiencies J Lab clin Med *68* 21 1966

127 *Jackson D P Morse E E Zieve, P D & Conley C L.* Thrombocytopathic purpura associated with defective clot retraction and absence of platelet fibrinogen Blood *22* 827 1963

128 *Jacobson B M* Effects of cortisone and corticotropin on prolonged bleeding time Arch intern Med *92* 471 1953

129 *Jerushalmy Z Skoza L Zucker M B & Grant R* Inhibition by guanidino compounds of platelet aggregation induced by adenosine diphosphate Biochem Pharmacol *15* 1791 1966

130 *Jerushalmy Z & Zucker M B* Some effects of fibrinogen degradation products (FDP) on blood platelets Thrombos Diathes haemorrh (Stuttg) *15* 413 1966

131 *Johnson S A Balboa, R. S Pederson H J & Buckley M* The ultrastructure of platelet participation in hemostasis Thrombos Diathes haemorrh (Stuttg) *13* 65 1965

132 *Johnson, S A, Monto R. W & Caldwell M J* A new approach to the thrombocytopathies Thrombocytopathy A Thrombos Diathes haemorrh (Stuttg) *2* 279 1958

133 *Johnson S R Bygdeman S & Eliasson R.* Effect of dextran on postoperative thrombosis Acta chir scand in press

134 *Jurgens R* Hereditare Thrombopathien V Kongress d. europ Ges Hämat Freiburg 1955 Springer Heidelberg 1956 p 396

135 *Kanska B Niewiarowski S Ostrowski L Poplanski A & Prokopowicz J* Macrothrombocytic thrombopathia Clinical coagulation and hereditary aspects Thrombos Diathes haemorrh (Stuttg) *10* [illegible] 1963

136 *Kaser Glanzmann R. & Lüscher E F* The mechanism of platelet aggregation in relation to hemostasis Thrombos Diathes haemorrh (Stuttg) *7* 480 1962

137 *Kendall A G Lowenstein L. & Morgen R. O* The hemorrhagic diathesis in renal disease (with special reference to acute uremia) Canad med Ass J *85* 405 1961

138 *Kloeze J* Influence of prostaglandin on platelet adhesiveness and platelet aggregation Proc Nobel Symposium II Stockholm 1966

139 *Koekenberg L. J L* Experimental use of macrodex as a prophylaxis against postoperative thromboembolism Bull Soc internat. Chir *21* 501 1962

140 *Kopec M Budzynski A. Stachurska J Wegrzynowicz Z & Kowalski E* Studies on the mechanism of interference by fibrinogen degradation products (FDP) with the platelet function Role of fibrinogen in the platelet atmosphere Thrombos Diathes haemorrh (Stuttg) *15* 476 1966

141 *Kowalski E. Budzynski A Z Kopec M Latallo Z. S Lipinski B & Wegrzynowicz Z* Studies on the molecular pathology and pathogenesis of bleeding in severe fibrino

lytic states in dogs Thrombos Diathes haemorrh (Stuttg) *12* *69*, 1964

142 *Kowalski E., Kopec M & Wegrzynowicz Z.* Influence of fibrinogen degradation products (FDP) on platelet aggregation adhesiveness and viscous metamorphosis Thrombos Diathes haemorrh (Stuttg) *10* 406 1963

143 *Kuhlback B* Bleeding tendency in chronic renal failure Acta med scand *157* 174 1957

144 *Langdell, R. D Adelson E. Furth F W & Crosby H* Dextran and prolonged bleeding time J amer med Ass *166* 346 1958

145 *Larrain, C & Adelson E* Hemostatic defect of uremia I Clinical investigation of three patients with acute post traumatic renal insufficiency Blood *11* 1059, 1956

146 *Larrieu M J* Congenital haemorrhagic disorders with normal platelet count and prolonged bleeding time Series haemat (Copenhagen) *7* 39 1965

147 *Larrieu M J Marder V J & Inceman S.* Effects of fibrinogen degradation products on platelets and coagulation Thrombos Diathes haemorrh (Stuttg) Suppl *20* 215 1966

148 *Lechner K & Deutsch E* Biochemical and ultrastructural findings in thrombasthenia Platelet conference Castel Miemo 1967

149 *LeRoy E. C Mason R. G & Brinkhous K M* Species differences in platelet agglutination in man and in the dog swine and rabbit Amer J Physiol *199* 183 1960

150 *Lewis J H Zucker M B & Ferguson J H* Bleeding tendency in uremia Blood *11* 1073 1956

151 *Luscher E. F* Viscous metamorphosis of blood platelets and clot retraction Vox sang *1* 133 1956

152 *Luscher E. F* Platelets in haemostasis and thrombosis Brit J Haemat. *13* 1 1967

153 *Macmillan D C* Secondary clumping effect in human citrated platelet rich plasma produced by ADP and adrenaline Nature Lond *211* 140 1966

154 *Marcus A J & Zucker M B* The physiology of blood platelets Grune & Stratton New York 1965

155 *Marx R & Jean G* Studien zur Pathogenese der Thrombasthenie Glanzmann Naegeli Klin Wschr *40* 942 1962

156 *Marchouf C Robinson R W & Le Beau R J* Evaluation of Nialamide on the coagulation of blood Blood *24* 289 1964

157 *Mason R G & Read M S* Platelet response to six agglutinating agents species similarities and differences Exper Mol Path *6* 370 1967

158 *McClure P D Ingram G I C Stacey R S Glass V H & Matchett M O* Platelet function tests in thrombocythaemia and thrombocytosis. Brit. J Haemat. *12* 478 1966

159 *McDonald L. & Edgill M* Action of heparin in ischaemic heart disease Lancet *I* 844 1961

160 *McLean J R Maxwell R. E. & Hertler D* Fibrinogen and adenosine diphosphate induced aggregation of platelets Nature Lond *202* 605 1964

161 *Meyer D & Larrieu M J* L analyse des fonctions plaquettaires Mesure de l adhesivite des plaquettes au verre sur sang total (Methode de Salzman) Rev franç Etud clin biol *12* 736 1967

162 *Meyer D Larrieu M J Caen J & Bernard J* Criteres et limites du diagnostic de la maladie de Willebrand Nouv Rev franç Hemat *7* 115 1967

163 *Millac P Caspary E. A & Irineas J* Atromid and platelet adhesiveness in multiple sclerosis J atheroscler Res *7* 397 1967

164 *Mitchell J R A & Sharp A A* Platelet clumping in vitro Brit J Haemat *10* 78 1964

165 *Moolten S E Vroman L. & Vroman G M S* Adhesiveness of blood platelets in thromboembolism and hemorrhagic disorders. II Diagnostic and prognostic significance of platelet adhesiveness Amer J clin. Path *19* 814 1949

166 *Morris C D W* Acetylsalicylic acid and platelet stickiness Lancet *I* 279 1967

167 *Movat, H Z Mustard J F Taichman N S & Uriuhara T* Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen antibody complexes Proc Soc exp Biol (N Y) *120 232 1965*

168 *Murer E H Hellem A J & Rozenberg M C* Energy metabolism and platelet function Scand J clin Lab Invest *19 280 1967*

169 *Mustard J F Glynn M F Nishizawa E E & Packham M A* Platelet surface interactions relationship to thrombosis and hemostasis Fed Proc *26 106 1967*

170 *Mustard J F, Rowsell H C Smythe H A Senyi A & Murphy E A* The effect of sulfinpyrazone on platelet economy and thrombus formation in rabbits Blood *29 859 1967*

171 *Nieuwarowski S & Thomas D P* Platelet aggregation by ADP and thrombin Nature Lond *212 1544 1966*

172 *Nilehn J E* Split products of fibrinogen after prolonged interaction with plasmin Thrombos Diathes haemorrh (Stuttg) *18 89 1967*

173 *Nilehn J E* Separation and estimation of split products of fibrinogen and fibrin in human serum Thrombos Diathes haemorrh (Stuttg) *18 487 1967*

174 *Nilehn J E* Influence of split products of fibrinogen on results of blood coagulation tests and platelet adhesiveness Scand J haemat *4 430 1967*

175 *Nilsson I M* v Willebrand s disease in Sweden Baldacci Symposium, Mallorca 1960

176 *Nilsson I M* Fibrinolysis and defibrination Acta chir scand in press

177 *Nilsson I M* unpublished results

178 *Nilsson I M & Bjorkman S E* Experiences with ε aminocaproic acid (ε ACA) in the treatment of profuse menstruation Acta med scand *177 445 1965*

179 *Nilsson I M Blomback B Blomback M & Stenn rud S* Kvinnlig hamofili och dess behandl ng med humant antihamofili globulin Nord Med *56 1654 1956*

180 *Nilsson I M & Blomback M* Von Willebrand s disease in Sweden — occurrence pathogenesis and treatment Thrombos Diathes haemorrh (Stuttg) Suppl 2 103 1962

181 *Nilsson I M Blomback M & Blomback B* V Willebrand s disease in Sweden Its pathogenesis and treatment Acta med scand *164 263 1959*

182 *Nilsson I M Blomback M & von Francken I* On an inherited autosomal haemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time Acta med scand *159 35 1957*

183 *Nilsson I M Blomback M Jorpes E Blomback, B & Johansson S A* v Willebrand s disease and its correction with human plasma fraction I—O Acta med scand *159 179 1957*

184 *Nilsson I M Blomback M & Ramgren O* Haemophilia in Sweden I Coagulation studies Acta med scand *170 665 1961*

185 *Nilsson I M Blomback M Ramgren O & Francken I* Haemophilia in Sweden II Carriers of haemophilia A and B Acta med scand *171 223 1962*

186 *Nilsson I M Blomback M Thilen A & v Francken I* Carriers of haemophilia A Acta med scand *165 351 1959*

187 *Nilsson I M & Cronberg S* A severe haemorrhagic disorder with prolonged bleeding time due to a plasma defect but with normal factor VIII Acta med scand in press

188 *Nilsson I M & Eiken O* Further studies on the effect of dextran of various molecular weight on the coagulation mechanism Thrombos Diathes haemorrh (Stuttg) *11 38 1964*

189 *Nilsson I M Magnusson S & Borchgrevink C* The Duke and Ivy methods for determination of the bleeding time Thrombos Diathes haemorrh (Stuttg) *10 223 1963*

190 *Nilsson I M Nilehn J E Cronberg S & Norden G* Hypofibrinogenaemia and massive thrombosis Acta med scand *180 65 1966*

191 *Nilsson I M & Olow B* Determination of fibrinogen and fibrinogenolytic activity

Thrombos Diathes haemorrh (Stuttg) 8 297 1962

192 *Nilsson I M & Olow, B* Fibrinolysis induced by streptokinase in man Acta chir scand. *123 247 1962*

193 *Nilsson I M, Skanse B Bjorkman, S. E. & Sern F* Platelet function in thrombocythemia. The effect of platelets and serotonin on serum potassium and bilirubin Acta med scand *167 353 1960*

194 *Nordoy A & Ödegaard A E.* The influence of citrate and heparin on the adhesiveness of rat platelets and human platelets measured in vitro Scand. J clin Lab Invest. *15 399 1963*

195 *Nygaard K K* A direct method of counting platelets in oxalated plasma Proc Mayo Clin *8 365 1933*

196 O *Brien J R* The adhesiveness of native *platelets and its prevention* J clin Path *14 140 1961*

197 O *Brien J R* Platelet aggregation Part II. Some results from a new method of study J clin Path *15 452 1962*

198 O *Brien J R* A comparison of platelet aggregation produced by seven compounds and a comparison of their inhibitors J clin. Path *17 275 1964*

199 O *Brien J R* Platelets a Portsmouth syndrome Lancet *2 258 1967*

200 O *Brien J R.* Platelet adhesiveness tests in uraemia Lancet *I 1058 1967*

201 O *Brien J R & Heywood J B* A comparison of platelet stickiness tests during an Atromid S trial Thrombos Diathes haemorrh (Stuttg) *16 768 1966*

202 O *Brien J R. & Heywood J B* Some interactions between human platelets and glass von Willebrand s disease compared with normal J clin Path *20 56 1967*

203 O *Brien J R Heywood J B & Heady J A* The quantitation of platelet aggregation induced by four compounds a study in relation to myocardial infarction Thrombos Diathes haemorrh. (Stuttg) *16 752 1966*

204 *Odegaard A E. Skalbegg B A & Hellem A J* ADP induced platelet adhesiveness as a diagnostic test in von Willebrand s disease Thrombos Diathes haemorrh (Stuttg) *11 23 1964*

205 *Odegaard A E. Skalbegg B A & Hellem A J* Investigation on adenosine diphospate (ADP) induced platelet adhesiveness in vitro Part III The inactivation of ADP *in plasma Thrombos Diathes. haemorrh* (Stuttg) *11 317 1964*

206 *Ottaviani P Mandelli F & Deriu L* La proprieta adesiva delle piastrine nell angioemofilia B Progr med *19 370 1963*

207 *Owren P A* Coronary thrombosis Its mechanism and possible prevention by linolenic acid Ann intern Med *63 167 1965*

208 *Pachter M R Johnson S A & Basinski D H* The effect of macroglobulins and their dissociation units on release of platelet factor 3 Thrombos Diathes. haemorrh (Stuttg) *3 501 1959*

209 *Packham M A Warrior E. S Glynn M F Senyi, A S & Mustard J F* Alteration of the response of platelets to surface stimuli by pyrazole compounds. J exp Med *126 171 1967*

210 *Perkins H A* Brief report Correction of the hemostatic defects in von Willebrand s disease Blood *30 375 1967*

211 *Pitney W R & Potter M* Retention of platelets by glass bead filters J clin Path *20 710 1967*

212 *Pittman M A. Jr & Graham J B* Glanzmann s thrombopathy an autosomal *recessive trait in one family* Amer J med Sci. *247 293 1964*

213 *Praga C & Cortellaro M* Creatinine and platelets in uraemia Lancet *I 899 1967*

214 *Quick A J* Salicylates and bleeding The aspirin tolerance test. Amer J med Sci *252 265 1966*

215 *Ramgren O Nilsson I M & Blombuck M* Haemophilia in Sweden IV Hereditary *investigations Acta med scand 171 759* 1962

216 *Rath C E. Maillard J A & Schreiner G E.* Bleeding tendency in uremia New Engl J Med *257 808 1957*

217 *Robinson R W* Platelet adhesiveness and aggregation with chlorophenoxyisobutyric ester Amer J med Sci *253 76 1967*

218 *Rod na i Jr N F Ma o i R G Pa ter J C & Bri kl o s K M* F br nogen — ts role n platelet agglut nat on and agglut nate stab l ty A study of congen tal af br nogenemia Lab Invest *15* 641 1966

219 *Roskam J* Erreurs anc ennes et perspec t ves nou elles dans l etude de l adhes on de l agregat on et de la metamorphose v s queuse des plaquettes facteurs d hemostase Thrombos D athes haemorrh (Stuttg) *10* 253 1963

220 *Ros er P Bertl oud S & Bous er C A* In v tro effects of d pyram dole on platelet adhes veness to glass A prelim nary com mun cat on Vox Sang *12* 300 1967

221 *Ro e berg M C & D ntenfa s L* Platelet aggregat on in Waldenstrom s macroglobu linaem a Thrombos D athes haemorrh (Stuttg) *14* 202 1965

222 *Ro enberg M C & Stor norke i H* Com par son of glass adhes veness and rate of aggregat on of blood platelets Scand J clin Lab Invest *19* 82 1967

223 *Ro ens ajn L & Medt T* Thrombocyto pathy and trans tory forms between throm bocytopathy and thrombocytopen a Acta haemat. *22* 255 1959

224 *Sal man E W* Measurement of platelet adhes veness A simple n v tro techn que demonstrat ng an abnormal ty n von Wil lebrand s d sea e J Lab cl n Med *62* 724 1963

225 *Sal n an E W* The lim tat ons of hepar n therapy after arter al reconstruct on Sur gery *57* 131 1965

226 *Sal n an E W* Personal commun cat on

227 *Sal an E W & Cl a be s D A* Inh b t on of ADP induced platelet aggregat on by subst tu ed am no ac ds Nature Lond *204* 698 1964

228 *Sal n a E W Cl a bers D A & Ners L L* In o porat on of lab lled nucleot des and aggrega on of human blood platelets Thrombos D athes haemorrh (Stuttg) *15* 52 1966

229 *Sal ma E W & Ne L L* Adhes eness of blood pla elets n urem a Thrombos D athes haemo rh Stuttg) *15* 84 1966

230 *Sa bar S S Zu fler A J & Smet G* In v vo and in v tro effects of polyvinyl pyrrol done on platelet adhes veness n hu man blood Lancet 2 917 1967

231 *Sch d H J Jackso D P & Co ley C L* Mechan sm of act on of thromb n on platelets J cl n Invest *41* 543 1962

232 *Sl er a J L & Wu el H A* Compa rat ve effects of glyceryl gu accolate and adenosine on nh b t on of ADP induced platelet aggregat on Amer J med Sc *254* 491 1967

233 *Sl uer J* to be publ shed

234 *Sl uer J Cronberg S & N ls o I M* Occurrence of arter oscleros s n von W lle brand s d sease Acta med scand *180* 475 1966

235 *Sjol n K E & V debaek A* Chr stmas factor def c ency and decreased cap llary res stance n a female w th haemorrhag c d athes s Dan med Bull *3* 85 1956

236 *Skalhegg B A Hellem A J & Odegaard A E* Invest gat ons on adenos ne d phos phate (ADP) nduced platelet adhes veness n v tro Part II Stud on the mechan sm Thrombos D athes haen orrh (Stuttg) *11* 305 1964

237 *S ythe H A Ogryslo M A Murphy E A & Mu ta d J F* The effect of sul f npyrazone (Anturan) on platelet economy an blood coagulat on n man Canad med Ass J *92* 818 1965

238 *Solu n N O* Influence of f brinogen on the aggregation of washed human blood platelets Proc Xth Congr Europ Soc Haemat. Stra bourg 1965 Part II Karger Basel/New York 1967 1101

239 *Solu n N O* Platelet aggregat on dur ng f br n polymer zat on Scand J cl n Lab Invest *18* 577 1966

240 *Solum N O & Stormo ke i H* Influence of f br nogen on the aggregat on of washed human blood platelets nduced by adenosine d pho phate thromb n collagen and adrena line Scand J cl n Lab In est *17* Suppl *84* 170 1965

241 *Soul er J P & Larr eu M J* Def c t en facteur ant hemoph l c B a ec allongement du temps de sa gnement Sang *28* 138 1957

242 *Spaet T H & Lejnieks I* Studies on the mechanism whereby platelets are clumped by adenosine diphosphate Thrombos Diathes haemorrh (Stuttg) *15* 36 1966

243 *Spaet T H & Zucker M B* Mechanism of platelet plug formation and role of adenosine diphosphate Amer J Physiol. *206* 1267 1964

244 *Spooner M & Meyer, O O* The effect of dicumarol (3 3' methylenbis 4 hydroxy coumarin) on platelet adhesiveness Amer J Physiol. *142* 279 1944

245 *Stormorken H Lund Rose A & Rørvik T O* Platelet adhesiveness to glass beads Methodological investigations using automatic platelet counting Scand J clin Lab Invest. *17* Suppl 84 183, 1965

246 *Strauss H S & Bloom G E* von Willebrand s disease Use of a platelet adhesiveness test in diagnosis and family investigation. New Engl J Med 273 171 1965

247 *Swank R. C Fellman J H & Hissen W* Aggregation of blood cells by 5 hydroxy tryptamine (serotonin) Circ Res *13* 392 1963

248 *Symons C de Toszeghi A & Cook I J Y* Effect of ethylchlorophenoxyisobutyrate with or without adrosterone on platelet stickiness Lancet *2* 233 1964

249 *Ulutin O N* The qualitative platelet diseases Henry Ford Hospital Internat Symposium on Blood Platelets Little Brown & Co Boston 1961

250 *Ulutin O N* Primary thrombocytopathy Israel J med Sci *1* 857 1965

251 *Vainer H & Caen J* Utilisation d un test photometrique pour l etude de l effet de l ADP sur les plaquettes sanguines Nouv Rev franç Hemat *3* 149 1963

252 *Vainer H & Caen J* A useful photometric test for the diagnosis of von Willebrand s disease J clin Path *17* 191 1964

253 *Voss D* Untersuchungen zur Gerinnsel retraktion VIIth Congr Internat Soc Haemat 1243 1958

254 *Weiss H J* Platelet aggregation adhesion and adenosine diphosphate release in thrombopathia (Platelet factor 3 deficiency) A comparison with Glanzmann s thrombasthenia and von Willebrand s disease Amer J Med *43* 570 1967

255 *Weiss H J* The effect of clinical dextran on platelet aggregation adhesion and ADP release in man in vivo and in vitro studies J Lab clin Med *69* 37 1967

256 *Weiss H J & Aledort L M* Impaired platelet connective tissue reaction in man after aspirin ingestion Lancet *2* 495 1967

257 *White J G, Kunst E Collhander M & Krivit W* Prolonged bleeding time in a patient with plasma thromboplastin antecedent deficiency Observations on correction of the bleeding time by platelet transfusion J Pediat *63* 1081 1963

258 *Wilkinson T S Black W S Sutterfield T C & Stone H H* Experimental prophylaxis and treatment of venous thrombosis with dextran *75* J surg Res *7* 464 1967

259 *von Willebrand E A* Hereditär pseudohemofili Finska Lakarsallsk Handl *68* *87* 1926

260 *von Willebrand E A* Über hereditäre Pseudohämophilie Acta med scand *76* 521 1931

261 *Willoughby M C N & Crouch S* An investigation of the haemorrhagic tendency in renal failure Brit J Haemat *7* 315 1961

262 *Wilson P McNicol G P & Douglas A S* Platelet abnormality in human scurvy Lancet *1* 975 1967

263 *Wilson P A McNicol C P & Douglas A S* Some effects of trypsin and streptokinase on platelet aggregation Thrombos Diathes haemorrh (Stuttg) *18* 66 1967

264 *Wright H P* The adhesiveness of blood platelets in normal subjects and with varying concentrations of anticoagulants J Path Bact *53* 255 1941

265 *Wright H P* The adhesiveness of blood platelets in rabbits treated with dicumarol J Path Bact. *57* 382 1945

266 *Wright H M P* Adhesiveness of blood platelets in haemophilia Lancet *1* 306 1946

267 *Zucker, M B* Platelet adhesion, release and aggregation Thrombos Diathes haemorrh (Stuttg) Suppl *13* 301, 1964

268 *Zucker M B & Borelli J* Viscous metamorphosis of blood platelets produced by thrombin Fed Proc *14* 168 1955

269 *Zucker M B & Borelli, J* Platelet viscous metamorphosis and clot retraction Fed Proc *16* 140 1957

270 *Zucker, M B & Borelli, J* Platelet clumping produced by connective tissue suspensions and by collagen Proc Soc exp Biol (N Y) *109* 779 1962

271 *Zucker M B & Peterson J* Serotonin platelet factor 3 activity and platelet aggregating agent released by adenosine diphosphate Abstr 3rd Conf on Platelets Oak Ridge Blood *30* 556 1967

272 *Zucifler A J* Impairment of platelet function and thrombus growth in reserpine treated rabbits J Lab clin Med *70* 16, 1967

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SUPPLEMENTUM 487

ANEMIA IN CHRONIC PYELONEPHRITIS AND IN RENAL FAILURE OF ANALGESIC ABUSERS

BY

JORMA FORSST

HELSINKI 1968

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SUPPLEMENTUM 487

FROM THE DEPARTMENT OF MEDICINE (HEAD PROFESSOR PEKKA BRUMMER, M.D.) UNIVERSITY HOSPITAL, TURKU, FINLAND

ANEMIA IN CHRONIC PYELONEPHRITIS AND IN RENAL FAILURE OF

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OLYTIC ANEMIA

Translated by Aino Wuolle

Oy Weilin+Goos Ab Tapiola 1968

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INTRODUCTION

As early as 1827 Richard Bright described anemia as an additional sign in chronic insufficiency of the kidneys Though this nephrogenic anemia has been known for such a long time there is as yet no entirely satisfactory explana tion for its pathogenesis Researches have been directed mainly towards two mech anisms leading to anemia On the one hand, the anemia has been ascribed to inhibition of bone marrow erythropoiesis, on the other to increased hemolysis Evidence in favour of each of these mechanisms has been put forward, but most investigators have concluded that anemia in chronic renal insuffic ency is a result of the combination of several fac tors

At the present time, chronic pyelonephritis is the most common of the diseases resulting in renal insufficiency Anemia is frequently one of the early symptoms Renal damage assumed to be due to phenacetin containing analgesic bears a close relationship to chronic pyelonephritis In 1953, Spuhler and Zol linger demonstrated simultaneous abuse of analgesics in a number of patients with pathologic anatomical evidence of chronic interstitial nephritis Later several epidemiological studies have revealed a good correlation between abuse of phenacetin containing analgesics and renal disease On pathologic anatomical exam ination phenacetin abusers have been found to present chronic interstitial ne phritis, of either sclerosing or destructive type, or of intermediate type A chronic non obstructive pyelonephritis can also be regarded mainly as a destructive chronic interstitial nephritis This being so the pathologic anatomical changes in chronic pyelonephritis are predominantly the same as those in so-called phenacetin nephropathy The patient groups with the respective diseases do not differ essentially except as regards the abuse of phenacetin containing drugs

Increased hemolysis obviously plays a part in nephrogenic anemia The mecha nism of this hemolysis, however has not been definitely clarified even though it was concluded from many studies that the cause of the reduction of erythrocyte life span is not in the red cell itself but there is an extracorpuscular factor or factors responsible for the hemolysis Brain and his co workers stated in 1962, that the mechanical hemolysis associated with microangiopathy is a factor inde pendent of the erythrocyte The part played by such a mechanism, in acute renal insufficiency especially has been fairly convincingly established The com bination of phenacetin abuse with renal insufficiency makes it more difficult to disclose the mechanism of nephrogenic anemia especially as far as concerns increased hemolysis since phenacetin containing analgesics alone can result in increased hemolysis

This study was designed to inquire into whether and in what way the mechanism of the anemia of renal insufficiency differs when comparing cases associated with phenacetin abuse and those with chronic pyelonephritis only Particular attention was paid to the part played by microangiopathic hemolytic anemia in the above conditions

REVIEW OF THE LITERATURE

ANEMIA AS THE SIGN OF CHRONIC RENAL FAILURE

Anemia is nearly always present in chronic renal insufficiency A normochromic normocytic anemia may be the first indication of renal failure (e g de Gruchy 1964) It is one of the early signs of chronic pyelonephritis (e g Schoen 1962) in which condition hemoglobin is recognized to be a sensitive indicator of incipient renal insufficiency (Kasanen and Salmi 1959)

Anemia occurs independently of the nature of the renal disease (Parsons and Ekola Strolberg 1933 Callen and Limarzi 1950 Effersoe 1958) and is usually aggravated with further progress of the renal disease (Townsend *et al* 1937 Callen and Limarzi 1950) It has been found that as a rule anemia is already present when serum urea exceeds 70 mg/100 ml and serum creatinine reaches the limit of 2 mg/100 ml (Bock and Thedering 1952) or phenol red excretion declines below 20 per cent/2 hrs also when the non protein nitrogen level increases above 70 mg/100 ml and urea and endogenous creatinine clearance declines below 10 ml/min (Kasanen and Kalliomaki 1957), but anemia is rare when the serum creatinine value is lower than 3 mg/100 ml (Effersoe 1958) It has even been claimed that anemia is almost independent of the grade of severity of the renal disease (Nordenson 1959 Yavorkovskii 1962) but correlates well with its duration (Nordenson 1959)

Exacerbation of the anemia occurs as the renal disease progresses, but generally stops when a certain level of anemia is reached (Effersoe 1958) Roscoe (1952) has shown that as the serum urea value increases from 50 mg/100 ml to 250 mg/100 ml hemoglobin is reduced by about 2 g/100 ml for each 50 mg/100 ml increase in urea but that the anemia no longer worsens with an increase of the urea value beyond 250 mg/100 ml Kasanen and Kalliomaki (1957) noted that anemia did not increase further as the non protein nitrogen reading exceeded 110 mg/100 ml and Effersoe (1958) that anemia was maintained at a definite level when serum creatinine rose above 6 mg/100 ml

MECHANISM OF ANEMIA

The mechanism of this anemia has been studied ever since it was first recognized that anemia is associated with chronic renal disease In the earliest studies the cause was thought to be hydremia (e g Brown and Roth 1922, Becher 1930) Hematuria was also considered to produce anemia (e g Becher 1930) However the role of each of these two conditions in the production of ane

mia is slight, and for instance in chronic nephritis, no correlation has been found between the grades of microscopic hematuria and of anemia (Brown and Roth 1922, Callen and Limarzi 1950)

The anemia in chronic renal insufficiency has been mainly ascribed to two factors, (1) disturbed erythropoiesis and (2) increased hemolysis

DISTURBANCE OF ERYTHROPOIESIS

DEPRESSION OF ERYTHROPOIESIS IN CHRONIC RENAL FAILURE

Ceconi reported, in 1906, that the anemia accompanying renal insufficiency is due to toxic inhibition of hemopoiesis (cited by Brown and Roth 1922) Indeed the degree of bone marrow erythropoiesis was found by some investigators to be decreased in chronic insufficiency of the kidneys (Lowinger 1938, Nordenson 1938, Faarup and Ohlsen 1943) These observations however, have not been borne out by subsequent reports Andereggen (1946) found, in a series of 38 patients with nephrogenic anemia, that the number of erythroblasts was normal in 48 per cent reduced in 28 per cent, and elevated in 24 per cent Even in the cases associated with hypoplasia or aplasia, these latter could not be correlated with anemia Callen and Limarzi (1950) studied 102 patients with various renal diseases including 44 with azotemia Of these 44 [illegible] per cent showed a hypercellular bone marrow and erythropoiesis was normal A quantitative decrease of erythropoiesis was only present if the blood non protein nitrogen was above 150 mg/100 ml Bone marrow aplasia was not found Similar results have been obtained later too (Bock and Thedering 1952 Bock *et al* 1962 b)

The discrepancy between the presence of anemia and the apparently adequate erythropoiesis, quantitatively, has been assumed to point towards a disturbance in the maturation of red cells or in their delivery into the blood stream in uremia (Andereggen 1946 Callen and Limarzi 1950)

Studies using radioactive iron have convincingly demonstrated the presence of disturbed erythropoiesis in chronic nephropathy Finch *et al* (1949) noted reduced utilization of radioactive iron in five uremic patients Joske and his coworkers (1956) formed the same conclusion in the case of 15 uremic patients A decrease in the utilization of radioactive iron, which points to a depression of erythropoiesis, has also been established in a number of later studies (Desforges and Dawson 1958 Loge *et al* 1958, Naets *et al* 1960 Ragen *et al* 1960 Esbach *et al* 1967, Takasugi and Imura 1967)

Uremic plasma has been found to inhibit the maturation of erythroblasts in tissue culture (Sacchetti 1953 Markson and Rennie 1956, Berman and Powsner 1959) and the incorporation of iron in the bone marrow is reported to be lowered by the action of uremic plasma (Thorup *et al* 1958, Erslev and Hughes 1960) Uremic cells develop normally in normal plasma (Berman and Powsner 1959) It was not possible however, in all studies, to show inhibition of the normal cellular development by uremia On the basis of autoradiographic researches, Markson and Moore (1962) found that the hemoglobin and DNA synthesis of normoblasts were not inhibited by uremic plasma Hadnagy *et al* (1965) studied the effect of bilateral nephrectomy on the mitotic activity of the small intestine and tongue epithelium of rats They noted that mitotic cell division remains unchanged regardless of the development of uremia, from which they concluded they had been able in directly to demonstrate that the toxic metabolites accumulating in the body in uremia are not the cause of the inhibition of erythroblast proliferation in the bone marrow

THE EFFECT OF RETENTION OF TOXIC PRODUCTS

The part played by the depression of erythropoiesis in nephrogenic anemia is widely recognized (Emerson and Burrows 1949, Loge *et al* 1950, 1958, Gormsen and Gjorup 1955, Joske *et al* 1956, Kaye 1958, Nordenson 1959), and this depression has been attributed to the metabolites retained in uremia (Brown and Roth 1922, Scarlett 1929, Parsons and Ekola Strolberg 1933, Nordenson 1938 Bock and Weyand 1939 Faarup and Ohlsen 1943, Andereggen 1946, Roscoe 1952, Loge *et al* 1958)

Although the importance of retained products in the origin of renal anemia is considered obvious it has not been possible to incriminate any specific factor The anemia could generally be correlated with nitrogenous waste products (Scarlett 1929, Faarup and Ohlsen 1943, Callen and Limarzi 1950, Hadnagy *et al* 1965) and with creatinine in particular (Brown and Roth 1923 Parsons and Ekola Strolberg 1933 Effersoe 1958, Kasanen 1958, Bock *et al* 1962 b) The anemia is also related to indican (Nylander 1935 Bock and Weyand 1939, Kasanen 1958) to phenols (Dunn *et al* 1958 Bock *et al* 1962 b) and to urea (Misske and Otto 1936) No correlation has been found to exist between uric acid and anemia (Misske and Otto 1936 Kasanen *et al* 1958) In none of the studies was there a good correlation between anemia and the retained substance studied In favour of an inhibiting effect of retained metabolites however are those studies in which dialysis was found to abolish the inhibition of erythropoiesis (von Dittrich and Sartorius 1960) and to increase the utilization of iron (Kurtides *et al* 1964, Mann *et al* 1965, Esbach *et al* 1967)

ABSENCE OF ERYTHROPOIETIN

As early as 1906, Carnot and Deflandre advanced the idea of a humoral regulation of erythropoiesis (cited by Linman and Bethell 1960) Studies dealing with the regulation of erythropoiesis, the methods used for demonstration of erythropoietic activity, and with the nature and mechanism of erythropoiesis have been described in extensive surveys (Linman and Bethell 1960, Remmele 1963) The factor active in humoral control of erythropoiesis has been referred to by the generally accepted term *erythropoietin* (Bonsdorff and Jalavisto 1949)

The possible importance of a lack of erythropoietin in causing renal anemia has been the subject of active research since Jacobson and his co-workers published their results in 1957 They stated that, if rats rabbits or mice were bilaterally nephrectomized, no erythropoietic activity could be demonstrated in their plasma after induction of anemia by various methods Bilateral ureteral ligation, however, reduced erythropoietic activity only slightly as compared with the control animals (Jacobson *et al* 1957 a, 1957 b, Goldwasser *et al* 1958 Jacobson *et al* 1959 a, 1959 b) The part played by the kidneys in the regulation of erythropoiesis has been demonstrated in several series of animal tests using various experimental conditions (Naets 1958 a, 1958 b 1960 a 1960 b Osnes 1958, Reissman *et al* 1960, Kuratowska *et al* 1961, Rosse and Waldmann 1962, Fisher *et al* 1965 Fisher and Langston 1967)

Gallagher *et al* (1958, 1959) showed that the serum of a uremic anemic patient has no erythropoietic activity measured as Fe^{59} utilization in rats The same finding has been reported later (Gallagher *et al* 1960, Penington 1961 Naets and Heuse 1962) While Brown (1965) could find no erythropoietin in 12 out of 14 patients with the anemia of uremia the erythropoietin level was increased in two Erythropoiesis in renal diseases is independent of physiological regulation mechanisms (Brown 1966) Renal transplantation has been found to increase the

erythropoietin level of serum (Abbrecht and Greene 1966)

The juxtaglomerular cells have been thought of as the possible site of erythropoietin formation in the kidneys because of the changes observed in the granulation of these cells in connection with bleeding and hemolysis (Osnes 1958, Hirashima and Takaku 1962) A cortical extract of kidney possesses more erythropoietic activity than does the medulla (Czernobilsky and Erslev 1966)

The extent to which the kidneys are responsible for the production of erythropoietin is not yet fully clear Erslev (1958) showed, in rabbits, that the erythropoietic factor is related to the metabolic changes of uremia rather than to the renal tissue itself there was no significant erythropoietic response in the plasma of uremic and anemic animals whether they had been made uremic by bilateral nephrectomy or by ureteral ligation Following bilateral nephrectomy of rabbits and dogs, erythroid concentration in bone marrow was normal or increased (Muirhead *et al* 1952 1953) and in rats nephrectomy did not prevent the increase of erythropoetic factor in the plasma when hypoxia was used as stimulus (Mirand and Prentice 1957, Mirand *et al* 1959) It has also been demonstrated that the erythropoietically active factor produced in the kidneys is not an erythropoietin proper (Osnes 1959), it was assumed that the renal erythropoietic factor only becomes active in combination with the factor residing in the serum or that the former acts as an enzyme in the formation of erythropoietin proper (Contrera *et al* 1966) According to Nathan *et al* (1964) erythropoiesis increased in response to hypoxia in an anephric patient

Despite certain conflicting results in studies on the relationship between erythropoietin and the kidneys it seems obvious that the kidneys are active in the control of erythropoiesis Of special interest in this respect are a number of studies in which polycythemia occurred in association with tumours of the kidney (Forssell 1958, Remmele 1963)

HEMOLYSIS

Increased hemolysis is also recognized as a cause of the anemia of chronic renal disease (Emerson and Burrows 1949 Bock and Thedering 1952, Chaplin and Mollison 1953, Sutherland *et al* 1955, Joske *et al* 1956 Desforges and Dawson 1958 Kaye 1958 Loge *et al* 1958, Verel *et al* 1959, Naets *et al* 1960, Rees *et al* 1960 Ragen *et al* 1960, Shaw 1967, Stewart 1967)

Reticulocytosis suggesting increased hemolysis occurs in part of the patients (Bock and Thedering 1952 Gormsen and Gjorup 1955 Loge *et al* 1958, Shaw and Scholes 1967) The number of reticulocytes increases with depression of hemoglobin and increasing azotemia (Shaw and Scholes 1967) An increase in the number of reticulocytes is not always present and for instance in Stewart s (1967) report the greater part of the patients had normal reticulocytosis and the number of reticulocytes was not related to the degree of anemia or azotemia

Shortening of the life span of erythrocytes is a feature associated with increased hemolysis In several studies, some or even nearly all of the patients with chronic renal disease showed a shortened red cell survival (Chaplin and Mollison 1953 Sutherland *et al* 1955 Joske *et al* 1956 Desforges and Dawson 1958 Kaye 1958 Loge *et al* 1958, Naets *et al* 1960, Ragen *et al* 1960 Shaw 1967 Stewart 1967) Chaplin and Mollison (1953) reported reduction of red cell survival time in 5 out of 6 patients in the terminal stages of chronic nephritis, and Sutherland *et al* (1955) in 9 out of 11 patients with chronic uremia The series of Desforges and

Dawson (1958) consisted of a total of 54 patients including 25 with chronic pyelonephritis, red cell survival was determined on 14 patients and was normal in 3 only Kaye (1958) calculated the erythrocyte survival for 10 patients, it was reduced in 5, all of them cases of chronic pyelonephritis The role of hemolysis in renal disease of various types was studied by Stewart (1967) one group consisted of 16 patients with chronic renal disease and eight of them had chronic pyelonephritis Erythrocyte survival was on an average shorter than normal

It has been claimed that hemolysis does not occur until the late stage of uremia (Emerson and Burrows 1949 Chaplin and Mollison 1953 Joske *et al* 1956, Loge *et al* 1958) and that it is a sign of poor prognosis (Joske *et al* 1956) However the results in regard to a correlation between red cell life span and azotemia are controversial The majority of studies have failed to reveal any association between azotemia and erythrocyte survival (Chaplin and Mollison 1953 Desforges and Dawson 1958 Verel *et al* 1959 Stewart 1967), but Shaw (1967) on his part found that red cell survival could be correlated with both serum creatinine and serum urea

The life span of the red blood cells is not related to the grade of anemia (Desforges and Dawson 1958) or the correlation is only reasonably good (Shaw 1967) This is understandable, as hemolysis alone has not been considered an adequate reason for anemia to supervene in renal patients depression of erythropoiesis is always present in addition (Emerson and Burrows 1949, Bock *et al* 1952 Chaplin and Mollison 1953, Joske *et al* 1956 Loge *et al* 1958 Rees *et al* 1960)

The study of the mechanism of hemolysis has centred upon whether hemolysis is due to a defect in the red cell itself (intracorpuscular hemolysis) or to a factor external to the cell (extracorpuscular hemolysis)

By animal experimentation it has been established that bilateral nephrectomy rapidly results in a hemolytic anemia which has been considered intracorpuscular (Muirhead *et al* 1952, 1953 Sutherland *et al* 1955) Normal renal tissue has proved to inhibit hemolysis (Muirhead and Jones 1963) Sutherland *et al* (1955) carried out a study in which red cells from a uremic dog had a reduced life span if they were infused into a healthy dog, their results point to intracorpuscular hemolysis The same observation was made in two cases by determining the survival, in a healthy subject, of red cells obtained from a uremic patient

Most reports, however, indicate that hemolysis is extracorpuscular Cross transfusion tests have shown that red cells from a uremic patient survive normally in a healthy subject while the survival of healthy red cells is reduced in a uremic patient (Joske *et al* 1956 Desforges and Dawson 1958, Kaye 1958 Loge *et al* 1958) Autohemolysis *in vitro* is increased in the blood of uremic patients, but it decreases significantly if the uremic red cells are incubated in normal plasma or if the uremic plasma is dialysed *in vitro* before incubation (Giovannetti *et al* 1965) Dialysis restores the survival of red cells to normal (Rees *et al* 1967)

Acidosis diminishes the activity of certain enzymes and this may be the reason why red cell survival is reduced in uremia (Bock *et al* 1962 a 1965) Sailer *et al* (1963) demonstrated a rapid diminution of red cell glucose 6 phosphate dehydrogenase activity in cases of anuria On the other hand, the same enzyme has proved to be increased in renal anemia in all or in part of the patients (Nikkila *et al* 1960 von Dittrich 1962, Vuopio 1963) but it has been explained as due to rejuvenation of red cell population as a result of hemolysis (von Dittrich 1962)

Osmotic resistance of the red cells is normal in renal anemia (Kaye 1958) or slightly abnormal in part of the cases (Desforges and Dawson 1958, Loge *et al* 1958) A slight increase in osmotic resistance was found to be unrelated to anemia azotemia or to mechanical fragility of erythrocytes (Desforges and Dawson 1958)

Mechanical red cell resistance is either normal (Loge *et al* 1958) or reduced in part of the cases (Desforges and Dawson 1958) The increase of fragility, however is related neither to the non protein nitrogen nor to the hematocrit value

According to the majority of studies reported, the anemia of renal disease is not an autoimmune hemolytic anemia The Coombs test is negative (Joske *et al* 1956, Kaye 1958, Loge *et al* 1958 Verel *et al* 1959) Yavorkovskii (1962), however, obtained positive Coombs tests in a great number of anemic patients with pyelonephritis

The serum haptoglobin level is reported to be reduced in those renal patients in whom red cell survival is decreased (Stewart 1967)

Reports concerned with the role of microangiopathy in the causation of renal anemia and with the incidence of hemolytic anemia in patients who have chronic renal disease and have used phenacetin-containing analgesics will be dealt with in a later section of this study

MICROANGIOPATHIC HEMOLYTIC ANEMIA (MHA)

GENERAL CONSIDERATIONS

In 1962 Brain *et al* published a paper in which they stated that the hemolytic anemia occurring in certain pathological conditions is due to simultaneous vascular changes The hemolysis results from mechanical injury of the red cells in pathological blood vessels They referred to this condition as microangiopathic hemolytic anemia which term was first used by Symmers (1952) when describing thrombotic thrombocytopenic purpura Symmers however did not find any causal connection between the vascular changes and the hemolytic anemia

Brain and his co-workers studied 120 patients 113 of whom had acute or chronic renal disease two thrombotic thrombocytopenic purpura without uremia and five disseminated carcinoma Twenty five of the patients had hemolytic anemia In the peripheral blood, the red cells of all these patients showed the same morphological changes as had earlier been referred to by some descriptive terms (burr cells helmet cells triangular cells irregularly crenated cells) The degree of this poikilocytosis was compared with the vascular changes observed and a good correlation was found The investigators concluded that in these cases the hemolytic anemia was due to the red cells being injured as a result of their contact with damaged blood vessels and that this anemia was characterized by specific poikilocytosis besides by other findings typical of hemolytic anemia

The pathogenesis of the above described microangiopathic hemolytic anemia also has the support of experimental investigations In rabbits, a generalized Schwartzmann reaction was found to lead to intravascular hemolysis Burr cells, frag-

mented red cells and spherocytes appeared in the blood (Brain 1963) Brain and Hourihane (1967) produced vascular kidney changes in rabbits by using endotoxin These changes correlated well with plasma hemoglobin values, and poikilocytosis was present in the blood Experimentally induced vascular thrombus causes intravascular hemolysis (Brain *et al* 1967)

Bell (1963) has described the origin of burr cells

In favour of the mechanical nature of microangiopathic hemolytic anemia is the fact that a similar poikilocytosis has been observed in other mechanically produced hemolytic anemias The question is most closely related to cardiology and cardiac surgery

Sayed and his co workers (1961) dealt with hemolytic anemia associated with fragmentation and morphological change of erythrocytes in a patient following closure of a septal atrial defect with a teflon graft The hemolysis disappeared when a second operation was performed and the teflon graft covered Red cell fragmentation was assumed to have been caused by the blood stream being directed against a bare teflon prosthesis

A hemolytic anemia of a similar kind has later been encountered in connection with the insertion of valvular prosthesis (Brodeur *et al* 1965, Sears and Crosby 1965, Rubinson *et al* 1966) with the closure of ostium primum defect (Furuhjelm *et al* 1964) in valvular defects (Miller *et al* 1966 Westring 1966, Ziperovich and Paley 1966), and in atrial myxoma (Nikkila and Vuopio 1964)

POIKILOCYTOSIS TYPICAL OF MHA IN VARIOUS PATHOLOGICAL STATES

Schwartz and Motto in 1949 called attention to the occurrence of poikilocytes with one or more spiny projections at the periphery They termed these cells "burr" cells and considered that their presence may provide the first clue to diagnosis Their patients included 75 with uremia Burr cells were present in 54 (73 per cent) of this number These cells accounted for from 0 1 per cent to 1 5 per cent of the total number of red cells Schwarz and Motto stated that no correlation existed between the incidence of burr cells and race, sex, age, height of blood pressure, the anemia, mean hemoglobin concentration, or the levels of non protein nitrogen or creatinine They studied 100 additional patients with some hematologic aberration and found burr cells in 21 All of these had renal impairment

Dacie (1954) described irregularly contracted red cells in several uremic patients Such cells were present especially if there were signs of hemolytic anemia

Aherne (1957) studied the incidence of burr cells in 25 patients with azotemia changes were found in 13 Only one of these had a blood urea level below 150 mg/100 ml In four of the 12 patients who had a normal blood picture, the blood urea level was increased

In the study of Brain *et al* (1962) referred to already, the above poikilocytosis was determined semiquantitatively in various kidney diseases The poikilocytosis was related to anemia ($r = -0\ 56$) but not to blood urea level Three of the patients studied had chronic pyelonephritis

Levanto and Forsstrom (1964) described typical MHA poikilocytosis in 10 patients affected with chronic renal diseases of different kinds

Stewart (1967) studied the hemolysis in various kidney disorders He demonstrated red cell fragmentation and burr cells in 32 of a total of 50 patients tested Eight of the total number had chronic pyelonephritis Seven of these eight proved to have a fairly marked poikilocytosis The pyelonephritis patients included two who were abusers of phenacetin containing analgesics one of them showed a

moderate poikilocytosis, the other one no changes The poikilocytosis was not found to differ in the different groups of renal diseases it was unrelated to the life span of erythrocytes but was related to the serum urea nitrogen level ($r = 0\ 56$)

Red cell fragmentation was demonstrated in the hemolytic-uremic syndrome in children (Gasser *et al* 1955, Allison 1957, Shumway and Miller 1957, Lock and Dormandy 1961)

As will have been seen from the foregoing MHA poikilocytosis has been observed mainly in association with renal diseases However, this kind of poikilocytosis has been disclosed in other diseases too In 1953 Dacie and his coworkers described atypical hemolytic anemias stating that deformed red cells were abundantly present in the blood of one patient The diagnosis was later established as thrombotic thrombocytopenic purpura, a condition reported to be associated with MHA poikilocytosis (e g Adelson *et al* 1954 McCormack *et al* 1963) MHA poikilocytosis has also been observed in carcinomatosis (Dacie 1954, Brain *et al* 1962), carcinoma of the stomach (Schwartz and Motto 1949, Lehtinen 1967 Lynch *et al* 1967) breast carcinoma (Propp 1966), eclampsia (Seftel and Metz 1957), malignant hypertension (Capelli *et al* 1966) and in hemangioma (Propp and Scharfman 1966)

CHRONIC ABUSE OF PHENACETIN CONTAINING ANALGESICS AND RENAL DISEASES

GENERAL CONSIDERATIONS

In their study published in 1953, Spuhler and Zollinger stated that cases of primary chronic interstitial nephritis coming to autopsy have increased and suggested the abuse of phenacetin-containing analgesics as a possible etiological factor In many countries interest has since been stimulated in the part played by analgesics in the development of renal disorders (Zollinger 1955, Tholen *et al* 1956, Gsell *et al* 1957 Harvald 1957 Moeschlin 1957 1958 Gsell 1958, Larsen and Møller 1959 Lindeneg *et al* 1959, Moolten and Smith 1960, Buchanan 1961, Kasanen and Salmi 1961, Lakey 1961 Nordenfelt and Ringertz 1961 Bengtsson 1962 Jacobs and Morris 1962 Levin *et al* 1962 Rapoport *et al* 1962, Fifield 1963 Reynolds and Edmondson 1963, Rubenstein *et al* 1964 Young *et al* 1965)

The designation interstitial nephritis covers all inflammatory processes, acute or chronic occurring mainly in the interstitial tissue of the kidneys (e g Spühler 1962) Several pathogenetic factors may be considered (Zollinger 1945, Tholen 1954 Spuhler 1962) Sclerosis of the interstitium dominates the pathologic anatomical picture and papillary necrosis is common in primary chronic interstitial nephritis (Spuhler and Zollinger 1953 Zollinger 1955, Uehlinger 1958, Gloor 1961) This sclerosing interstitial nephritis (Gloor 1961) differs from non-obstructive chronic pyelonephritis in which the changes though also predominantly interstitial are of destructive nature (Gloor 1961) Papillary necrosis occurs in this latter also but to a less extent than in the sclerosing type (Gloor 1961) A chronic non-obstructive pyelonephritis can also be referred to as destructive chronic interstitial nephritis (Spuhler

1960) Chronic interstitial nephritis may be of a type intermediate between the sclerosing and destructive types (Thiel *et al* 1964)

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PHENACETIN AS THE CAUSE OF RENAL DISEASE

The literature dealing with the importance of phenacetin in the origin of renal disease has increased greatly over the years Attempts have been made by various methods to disclose the possible causal connection between phenacetin and kidney disease, animal tests have been carried out to study the effect of phenacetin on the kidney with a view to revealing the mechanism of the possible renal disorder

As already stated, chronic interstitial nephritis and papillary necrosis have increased during the last decades Concurrently, there has been a powerful increase in the consumption of phenacetin in many countries (e g Gsell 1958, Pletscher 1958, Harvald and Clausen 1960 Gloor 1962, Schreiner 1962)

Epidemiological researches have established a correlation between the abuse of analgesics and renal disease Larsen and Moller (1959) found renal function to be reduced in 33 2 per cent of the patients who had used analgesics daily Patients who had taken no analgesics included only 8 6 per cent with depressed renal function Kasanen and Salmi (1961) stated that regular abuse of analgesics was significantly greater among patients with renal disease than among other patients Grimlund s (1963) report is worth special notice, he found that mortality from uremia at Huskvarna, Sweden was significantly higher than at Fagersta, used for control purposes and correspondingly the consumption of phenacetin in the former was ten times as great as in the latter There are also other investigations pointing to a similar correlation (Horisberger *et al* 1958, Clausen and Pedersen 1961)

The correlation between phenacetin abuse and renal disease has not been universally accepted, however In Reubi s (1958) view, the simultaneous occurrence of phenacetin abuse and renal disease is due to chance, or the abuse is a result of the renal condition Sørensen (1960, 1963, 1965, 1966) failed to demonstrate any relationship between phenacetin abuse and renal disease

Animal tests have been widely used to determine the possible effect of phenacetin and analgesic compounds on the kidneys. Tholen *et al.* (1956) caused inflammatory interstitial changes in mice by feeding them a phenacetin containing analgesic, Sandon[R], for two months. Interstitial changes have been produced in rats with phenacetin and with N-acetyl-p-aminophenol (Eisalo and Talanti 1961) and in rabbits with phenacetin and acetylsalicylic acid (Clausen 1964, 1967). Abrahams *et al.* (1964) fed rats phenacetin, alone or combined with acetylsalicylic acid and caffeine. The phenacetin group showed a higher incidence of serious renal disease. A combination of all three drugs caused very pronounced papillary changes. According to Studer and Zbinden (1955) and Schmal (1958) rats failed to reveal interstitial nephritis after phenacetin feeding. This also applies to rabbits (Gsell *et al.* 1957, Reubi 1958). Schnitzer *et al.* (1965) produced histological changes in rat kidney with phenacetin contaminated by acetic-4-chloranilide, but not with phenacetin alone: interstitial nephritis or papillary necrosis was not seen, however.

Phenacetin administration with simultaneous infection has been found of importance in the production of renal changes. Miescher *et al.* (1958) produced interstitial nephritis in rabbits by intravenous administration of *E. coli* suspension and feeding phenacetin at the same time. Neither *E. coli* injection nor phenacetin alone sufficed to cause changes. There have been reports of similar findings later (Angervall *et al.* 1962). *B. proteus* injections accompanied with phenacetin administration, it is true, had a transitorily increasing effect on blood urea, but there were no histological changes (Miescher and Studer 1961). Clausen (1967) reported, that administration of phenacetin and acetylsalicylic acid to rabbits leads to direct toxic damage of the renal tissue and that secondary, intravenously inflicted *E. coli* infection has no share in the development of the characteristic renal changes. Nor was any difference noted between the kidneys of control rats and those fed on phenacetin when both groups were infected with an intravesical *E. coli* injection (Dawborn *et al.* 1964).

Phenacetin ingestion has been stated to result in changes in the metabolism of tubular cells in mice (Frey 1958), to decrease the concentrating capacity of the kidneys in rats (Angervall *et al.* 1964), to augment the excretion of acid mucopolysaccharides in rats (Vanto *et al.* 1964), and to cause histological changes in the tubular cells (Schourup *et al.* 1963, Fordham *et al.* 1965). A hematogenic interstitial nephritis induced artificially by means of staphylococci has been found to be aggravated in rats, by phenacetin administration (Studer *et al.* 1958 a), whereas some other artificially caused renal injuries were not aggravated, in rabbits and rats, by the use of phenacetin (Studer *et al.* 1958 b).

Using Addis count, Harvald and his co-workers (1960) demonstrated in patients that the count of erythrocytes in urine increased if, during the test period, the patient received phenacetin contaminated with acetic-4-acid - chloranilide. Later, however, Clausen and Harvald (1961) obtained similar results with acetylsalicylic acid, phenylbutazon and acetanilide, from which they concluded that the renal irritation was unspecific. Prescott (1965) arrived at the conclusion that the excretion of the tubular cells of the kidneys increases under the influence of phenacetin and acetylsalicylic acid. Veraguth and Spuhler (1961, cited by Thiel *et al.* 1964) have stated that phenacetin causes a disturbance in the excretion of amino acids in the urine, but concentrating capacity and phenol red remain unchanged. The excretion of acid mucopolysaccharides, both qualitative and quantitative, undergoes changes in patients using phenacetin (Kasanen *et al.* 1964).

Experimental investigations have failed

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creased hemolysis, but no poikilocytosis (Kasanen *et al* 1967) In cases in which hemolysis has resulted from the use of analgesic compounds, phenacetin alone has been found responsible (Miescher and Pletscher 1958) Hemolytic anemia evidently is fairly rare in phenacetin abusers Marti's (1958) study of 70 chronic phenacetin abusers included only two with demontrable hemolytic anemia and the degree of anemia bore no relationship to the amount of phenacetin taken Horsberger *et al* (1958) studied factory workers finding that phenacetin abusers did not differ from non abusers as regards hemoglobin content A healthy bone marrow is capable of compensating a phenacetin induced hemolysis if there are no simultaneous renal injuries (Miescher and Pletscher 1958)

Investigators of renal anemia in abusers of phenacetin containing analgesics have given chief attention to the part played by hemolysis in the origin of anemia Erythrocyte survival time has proved shorter in renal patients who have used phenacetin (Gsell *et al* 1957, Lorenzen and Schwarz 1960) Friis *et al* (1959) demonstrated that hemolytic anemia was more frequent in renal patients who were phenacetin abusers than in non abusers Later, they (Friis *et al* 1960) showed that red cells from a phenacetin addicted renal patient when reinjected had a normal lifetime in a healthy subject, whereas red cells from a healthy person survived a shorter time in phenacetin abusing patients with renal disease They attributed this phenomenon to the serum of the renal patients Nissen and Friis (1962) stated that, in phenacetin addicts with renal insufficiency, erythrocyte survival time was only shortened if further phenacetin was administered during the test Renal insufficiency by itself was not found to reduce erythrocyte survival time The hemolysis has been accounted for by a sensitizing mechanism (Friis *et al* 1960, Lorenzen and Schwarz 1960) There is no significant difference between ordinary purified phenacetin and a phenacetin containing acetic-4 chloranilide as impurity as fas as their hemolysis inducing effect is concerned (Friis and Nissen 1963)

The anemia of patients with renal insufficiency would be expected to become more severe with the added effect of phenacetin abuse The anemia, however, has been found to be equally severe in phenacetin abusers and non abusers with renal insufficiency if the severity of this insufficiency is the same in both groups (Thiel *et al* 1964) A Swedish report indicates that hemoglobin concentrations in renal patients were on an average higher after phenacetin consumption in Sweden had decreased (Bengtsson and Hood 1965) In some individual cases the anemia was corrected when the patient stopped taking phenacetin (Kasanen 1967)

OBJECT OF THE PRESENT INVESTIGATION

The aim of the present investigation has been to study possible differences as regards the mechanism of nephrogenic anemia among the following groups of patients

(1) Patients with diagnosed chronic pyelonephritis,
(2) patients with diagnosed chronic pyelonephritis and associated chronic abuse of phenacetin containing drugs,
(3) patients having chronic renal insufficiency and accompanying chronic abuse of phenacetin-containing analgesics but without clinically diagnosed chronic urinary infection

It was the purpose, in the case of the phenacetin abusers, to describe and illustrate the effect exerted on the anemia by the duration of the abuse and by its discontinuation

The poikilocytosis characterizing microangiopathic hemolytic anemia (MHA) was studied to determine the part played by it in the above three patient groups, and to see whether the poikilocytosis can be correlated with the anemia and the severity of the renal insufficiency and whether MHA poikilocytosis can be correlated with certain other findings indicative of increased hemolysis

MATERIAL

The study is based on 93 patients with chronic insufficiency of the kidneys treated in the Medical Department, University of Turku

The series consisted of three groups, as follows

Group I 27 patients with chronic pyelonephritis

Group II 42 patients with chronic pyelonephritis with a history of prolonged regular abuse of phenacetin-containing analgesics

Group III 24 patients with chronic renal insufficiency attributed to abuse of phenacetin containing analgesics in whom however chronic urinary infection had *not* been diagnosed

The distribution of the cases by age and sex is presented in Table 1

All patients had chronic renal insufficiency and those in groups II and III had, in addition, a clinically verified chronic pyelonephritis Seven patients of group I and 14 of group II were autopsied Papillary necrosis was found in three patients of the former group and nine of the latter

KIDNEY FUNCTION

Group I In 20 patients or 74 1 per cent the serum creatinine content was increased (>1 3 mg/100 ml), the mean (±S D) being 3 54±2 82 mg/100 ml

The specific gravity of morning urine was found to be low (≦1015) in all patients

Phenol red excretion was determined in 19 patients and found to be reduced (<55 %/2 h) in 16

In two of the patients in this group, *the decision as to renal insufficiency was* based only on the low specific gravity of morning urine

Group II 35 patients or 83 3 per cent showed an increased serum creatinine content, the mean being 4 05±3 46 mg/100 ml

In all patients, the specific gravity of morning urine was low

Table 1 *Distribution of cases according to age and sex Age range in brackets*

Group	Number	Men	Women	Age (mean)		
				Total	Men	Women
I	27	[illegible]	21	57 6 (35–79)	65 3 (52–75)	55.3 (35–79)
II	42	12	30	57 9 (40–79)	55 6 (44–70)	58.8 (40–79)
III	24	14	10	55 6 (41–72)	55 9 (41–70)	55 2 (42–72)
Total	93	32	[illegible]	57 2 (35–79)	57 5 (41–75)	57 0 (35–79)

Phenol red excretion was tested in 30 cases and found to be reduced in 27

In three of the patients in this group, renal insufficiency was considered to be present only on the basis of the low specific gravity of morning urine

Group III In 23 patients or 95 8 per cent the serum creatinine content was increased with a mean of 5 54±3 80 mg/100 ml

In all patients the specific gravity of morning urine was low

Phenol red excretion was studied in 14 patients and was reduced in 13 of them

The decision as to renal insufficiency was made in none of the cases on the basis of the specific gravity of morning urine only

URINARY FINDINGS

All patients of group I, and all except the five autopsied patients in group II, had had a previous urinary tract infection verified by laboratory tests In group III no urinary infections had been established earlier

The urinary findings in the various groups were as follows

Group I In 24 patients or 88 9 per cent there was pyuria (≥6 leucocytes in the high power field of the urinary sediment) on the occasion of examination None of the patients was found to have gross hematuria. Microscopic hematuria (>2 erythrocytes in high power field) was demonstrated in 13 patients or 48 0 per cent

Bacteria were cultured from the urine of 25 patients or 92 6 per cent In the case of two additional patients, the urine of one had earlier been positive on culture and the other was autopsied Bacterial counts were performed from the urine of 21 patients In 18 of these, the count was significant (>10 ml) two of the remaining three were under antibacterial medication and the third was autopsied

Group II Pyuria was present on examination in 32 patients or 76 2 per cent. Gross hematuria was found in none, microscopic hematuria in 22 patients or 52 4 per cent.

In 27 patients or 64 3 per cent cultures of urine were positive for bacteria Of the remaining 15 patients, five came to autopsy, five had earlier had positive cultures, and five were under antibacterial medication at the time of testing, but staining for bacteria from the sediment was positive Counting of bacteria from the urine was carried out in 24 cases, in 20 the count was significant, and 2 patients later showed a significant count

Group III 10 patients or 41 7 per cent had pyuria at the time of examination and 10 or 41 7 per cent had microscopic hematuria Gross hematuria was present in none

Staining of urinary sediment for bacteria was positive in 7 patients, in six of these cases bacteria were also cultured from the urine In 5 patients bacteria were counted, and three of these revealed significant counts

The borderline between groups II and III is not sharply defined Abuse of phenacetin-containing drugs was the common feature of both The clinical picture in the former group was dominated by recurrent urinary infection whereas no such infections had occurred in group III

ROENTGENOLOGIC FINDINGS

Azotemia prevented intravenous urography in many patients and it was performed in 17 cases Eight of these were group I cases and 9 group II cases All except one patient, belonging to group II, showed signs suggesting chronic pyelonephritis One of the patients in group II presented a change pointing to papillary necrosis

ABUSE OF ANALGESICS

Data on ingestion of analgesics are based on the patients' own statements. They were inquired about whether they had taken phenacetin-containing drugs and asked to evaluate the amount taken and the duration of abuse. The drug most commonly taken had the following composition: phenazon 0.5, phenacetin 0.4, caffeine 0.1. The patient was considered to have used analgesics regularly if he or she had taken the above or some corresponding drug daily or at least several times weekly for at least a year. Of the patients in groups II and III, 15 had used analgesics for over ten years, 43 for three to ten years, and only 8 for less than three years; these last 8 included only one who had taken analgesics for less than two years.

Following diagnosis of renal disease 20 patients had stopped taking these drugs two months to four years before the present investigation was started.

METHODS

GENERAL CONSIDERATIONS

The patients were accepted into this study on the basis of clinical examination and the principal disease in all cases was chronic insufficiency of the kidneys If a patient was anemic all causes of anemia unrelated to renal insufficiency were as far as possible excluded None of the patients had had any hemorrhage sufficient to account for the anemia Neither did anyone suffer from diseases frequently associated with symptomatic anemia (e g rheumatoid arthritis malignancies) If a patient was admitted with acute symptoms such as fever or definite disturbances of fluid balance he or she was treated to eliminate the acute symptoms before starting the investigations connected with the present study The patients had received no blood transfusions during the last few weeks preceding the examinations

LABORATORY EXAMINATIONS

The specific gravity of morning urine was determined on at least three successive mornings and the maximum value selected

Serum creatinine was tested by the method [illegible] Owen *et al* (1954)

[illegible]henol red excretion was determined by [illegible] the patient after emptying the [illegible] an intravenous 1 ml injection of [illegible] cent phenolsulfonphthalein and [illegible]ing the amount secreted into the [illegible]ing two hours

Th[illegible] sediment was studied from midstr[illegible]ne sample obtained after washing [illegible]ernal genitalia with water

Bacterial culture and counts were carried out immediately in the Department of Microbiology University of Turku For the colony count the sample was cultured with a calibrated platinum loop on a nutrient agar plate

Hemoglobin concentration hematocrit reading and the number of erythrocytes reticulocytes leucocytes and platelets were determined using conventional laboratory methods (Dacie and Lewis 1963)

MHA POIKILOCYTES

To study poikilocytosis a blood sample was withdrawn and a thin smear prepared The smear was stained by the method of May Grunwald Giemsa and examined under high power An area in the smear was chosen where the red cells were sufficiently far apart for demonstration of morphological changes Burr cells helmet cells triangular shaped cells irregularly crenated cells deformed spherocytes and fragmented erythrocytes (schistocytes) were interpreted as MHA poikilocytosis (examples shown in Figs 1—4) Regular shaped spherocytes were not taken into account Special efforts were made to avoid possible artefacts being included As such were regarded regularly spiculed cells and regularly crenated cells as well as the cells damaged during preparation of the smears

Quantitative estimation of poikilocytosis was carried out using balanced sampling (Woolf 1950, Brecher and Schneiderman 1950) An adjustable square diaphragm was utilized in microscopic examinations Visual field size was so

chosen that each field incorporated 20—30 red cells. Of the cells situated at the edges of the visual field those on the left and upper borders were included. An average of 10,000 red cells were counted from each smear.

It was concluded on the basis of quantitative determination that, if the rate of poikilocytosis is 1.0 per cent or higher poikilocytosis is easy to demonstrate even by routine methods. This limit was also used for appraising the degree of poikilocytosis when dealing with the results.

MHA poikilocytosis in health

MHA poikilocytosis is seldom encountered in healthy subjects (Schwartz and Motto 1949). For the purposes of the present investigation 30 successive blood donors[1] healthy in their own opinion, were studied and poikilocytosis was found as follows

No.	Sex	Age	Poikilocytosis
1	m	42 y	0.09 %
2	m	20 y	0.25 %
3	f	36 y	0.61 %
4	m	41 y	0.03 %
5	m	35 y	0.05 %
6	m	65 y	0.09 %
7	m	61 y	0.02 %
8	m	48 y	0.11 %
9	m	25 y	0.05 %
10	m	41 y	0.08 %
11	m	34 y	0.19 %
12	m	24 y	0.02 %
13	m	31 y	0.29 %
14	m	[illegible] y	0.01 %
15	f	38 y	0.01 %
16	m	42 y	0.13 %
17	f	22 y	0.05 %
18	f	45 y	0.49 %
19	m	40 y	0.07 %
20	m	34 y	0.06 %
21	m	62 y	0.07 %
22	f	19 y	0.01 %
23	m	37 y	0.08 %
24	m	30 y	0.10 %
25	m	53 y	0.02 %
26	f	74 y	1.20 %
27	f	56 y	0.02 %
28	f	52 y	0.03 %
29	f	23 y	0.04 %
30	f	51 y	0.05 %

The above control group thus consisted of 20 men and 10 women. Average age was 40.6 years (range 19—74 years). Study of the smears showed definite MHA poikilocytosis in one subject (No. 26) and quantitative determination resulted in the figure 1.20 per cent. On closer inquiry it appeared that the donor concerned had been under hospital treatment the year before, the diagnosis being Diabetes mellitus, arterial hypertension and pulmonary emphysema. In addition the patient had sustained a slight cerebral injury and showed signs of unilateral paresis. The serum creatinine content had been 0.9 mg/100 ml. The specific gravities of morning urine had been followed and several low values recorded in spite of the presence of sugar in the urine. This woman died of cerebral thrombosis in 1966.

Since one of the control subjects was ill, her MHA poikilocytosis being assumed to be due to vascular changes, the final number of controls was 29. For these the mean MHA poikilocytosis value was 0.11±0.14 per cent.

Osmotic resistance of the red cells was determined from whole blood without incubation by the method of Parpart *et al.* (1947).

The life span of erythrocytes was estimated using radiochromium labelled red cells (Dacie and Lewis 1963). By this method, $T^1/_2Cr^{51}$ varies from 25 to 32 days.

Serum iron was determined according to Trinder (1956) after correcting background absorbance by the method of Henry *et al.* (1958). Magnesium carbonate was used for TIBC determination.

Haptoglobin levels were estimated by means of Sephadex G 100 gel filtration (Juva and Laurent 1963).

Antiglobulin antibodies were determined by the Coombs test (1945).

Serum bilirubin values were measured using the method of Lathe and Ruthven (1958).

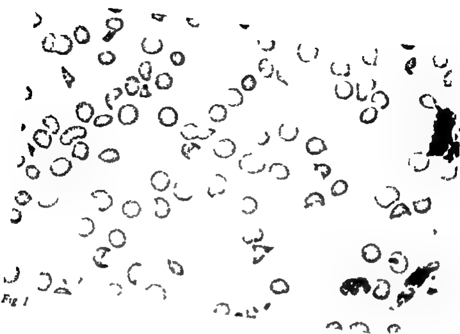
BONE MARROW

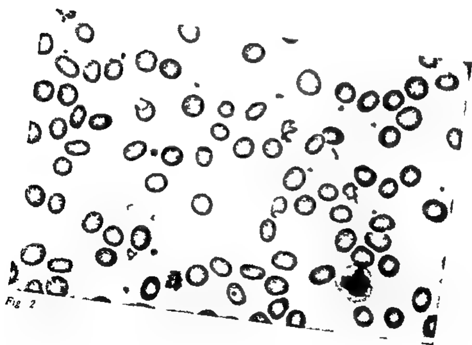
A sample was aspirated from the sternum for bone marrow studies. Both a smear and a pressure smear were prepared from a sample containing marrow particles. The preparations were stained by the May Grunwald Giemsa method and examined in the usual way. Special attention was paid to the degree of cellularity and the rate of erythropoiesis.

On the basis of cellularity the samples were divided into three categories and the following criteria used in estimations (Rohr 1960).

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[1] I thank The *Finnish Red Cross Transfusion* Centre Turku for collecting the control samples.


Fig 1


Fig 2

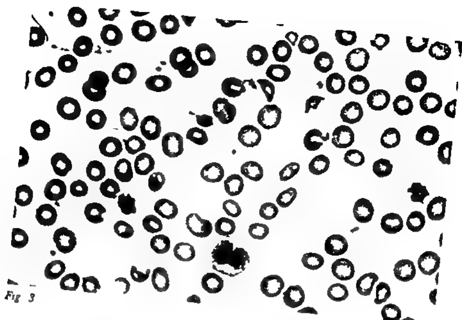


Fig 3

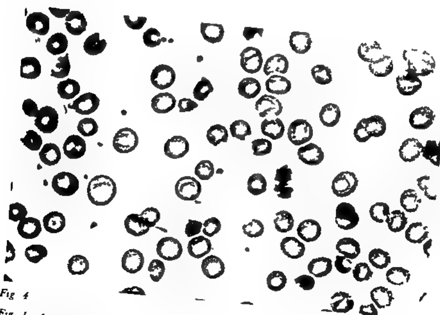


Fig 4

Fig 1—4 Photomicrographs of MHA poikilocytosis Burr cells helmet cells triangular shaped cells irregularly crenated cells deformed spherocytes x700

1 *Cellularity reduced* Fat spaces in bone marrow large, reticular cells predominate, few hematopoietic cells present

2 *Cellularity normal* Hematopoietic cells are spread evenly over the bone marrow, the number of fat spaces equals that of the spaces occupied by hematopoietic cells

3 *Cellularity increased* The bone marrow is almost filled with hematopoietic cells, fat spaces are small or absent

In judging the rate of erythropoiesis the samples were also divided into three classes according to the following criteria (Levanto 1966)

1 *Erythropoiesis reduced* Erythroblasts are not present in every visual field, the myeloid erythroid ratio is over 10 1

2 *Erythropoiesis normal* Erythroblasts are seen in each visual field, the myeloid-erythroid ratio is 10 1 or less

3 *Erythropoiesis increased* Erythroblasts are seen in abundance in each visual field There are also erythroblast groups (erythrons)

RESULTS

BLOOD

HEMOGLOBIN, HEMATOCRIT AND ERYTHROCYTES

In Table 2 are given the hemoglobin, hematocrit and erythrocyte values in the various groups and the total series.

Eighty-two of the patients in this study, or 88.2 per cent, were anemic (hemoglobin concentration below 11.5 g/100 ml in women and below 13.5 g/100 ml in men). Twenty patients had a hemoglobin value below 8.0 g/100 ml, but only 2 had severe anemia (hemoglobin below 6.0 g/100 ml). Of these last two one was in group II, the other in group III.

As will be seen in Table 2, group III showed less anemia but there was no significant difference between the groups as regards severity of anemia.

The anemia was normochromic in 57 patients or 69.5 per cent, hypochromic in 9 patients or 11.0 per cent, and mildly hyperchromic in 16 or 19.5 per cent. No inter-group differences were detected.

Table 2. *Hemoglobin, hematocrit and erythrocyte values in the various groups and total series*

	Group I	Group II	Group III	Total
Hemoglobin g/100 ml				
Mean	9.38	9.39	10.17	9.59
Range	6.7–12.2	5.5–15.2	5.5–13.6	5.5–15.2
S.D.	± 1.41	± 2.26	± 2.11	± 2.01
S.E.M.	± 0.27	± 0.35	± 0.43	± 0.21
n	27	42	24	93
Hematocrit %				
Mean	30.95	31.00	32.94	31.47
Range	20–40	17–48	17–46	17–48
S.D.	± 5.52	± 7.28	± 7.87	± 7.00
S.E.M.	± 1.20	± 1.21	± 1.81	± 0.80
n	21	36	19	76
Erythrocytes/mm³				
Mean	3.12	3.17	3.30	3.19
Range	1.9–4.3	1.7–4.9	1.8–4.7	1.7–4.9
S.D.	± 0.60	± 0.77	± 0.65	± 0.69
S.E.M.	± 0.12	± 0.12	± 0.14	± 0.07
n	27	42	21	90

RETICULOCYTES

The number of reticulocytes was recorded in 90 patients To evaluate the degree of reticulocytosis, the cases were divided into three groups The first consisted of those patients who had a normal reticulocyte count (≷2 0 per cent), the second of cases with slightly increased reticulocytosis (2 1—3 0 per cent), and the third of those with definite reticulocytosis (>3 0 per cent)

Table 3 shows the reticulocyte counts in the three groups and the total series and the classification according to degree of reticulocytosis It was found that the mean reticulocyte counts were higher than normal in all groups but intergroup differences were very slight

Increased values occurred in 49 patients of the total, or 54 4 per cent The distribution into groups was as follows group I 14 patients (52 per cent), group II 24 patients (59 per cent), group III 11 patients (50 per cent) Reticulocytosis exceeding 3 0 per cent was found in 32 patients or 35 5 per cent These last cases were most frequent in group I, though the differences between the three groups were not significant Reticulocyte counts exceeding 10 0 per cent occurred in only 3 patients, two in group II and one in group III

The degree of reticulocytosis increased with an increase in the severity of anemia The correlation between hemoglobin and reticulocytosis in the whole study was

$r = -0\ 33$ ($P<0\ 01$, $t = 3\ 25$)

The correlation varied slightly from one group to another, as follows

Group I $r = -0\ 50$ ($P<0\ 01$, $t = 2\ 92$)
Group II $r = -0\ 21$ ($P>0\ 05$)
Group III $r = -0\ 44$ ($P<0\ 05$, $t=2\ 16$)

MHA POIKILOCYTES

OVERALL FREQUENCY IN VARIOUS GROUPS CORRELATION WITH AGE AND SEX

The incidence of erythrocytes meeting the criteria for MHA poikilocytosis varied from 0 to 7 5 per cent The values in the various groups and in the total series are given in Table 4

It was found that all means differed very highly significantly ($P<0\ 001$) from the mean of the control series Intergroup differences were present The slightest MHA poikilocytosis occurred in

Table 3 *Reticulocyte counts (%) and classification according to degree of reticulocytosis in various groups and total series*

	Group I	Group II	Group III	Total
Mean	3 41	3 43	3 23	3 37
Range	0 4—9 8	0 4—13 4	0 6—11 0	0 4—13 4
S D	± 2 76	± 3 01	± 2 67	± 2 86
S E M	± 0 53	± 0 47	± 0 57	± 0 30
n	27	41	22	90
—2 0 %	13	17	11	41
	(48 %)	(42 %)	(50 %)	45 6 %
2 1—3 0 %	3	11	3	17
	(11 %)	(27 %)	(14 %)	18 9 %
3 1— %	11	13	8	32
	(41 %)	(31 %)	(36 %)	35 5 %

Table 4 *MHA poikilocyte counts (%) and classification according to degree of MHA poikilocytosis in various groups and total series*

	Group I	Group II	Group III	Total
Mean	0 63	1 62	1 80	1 38
Range	0–2 0	0 1–7 4	0–7.5	0–7 5
S D	± 0 54	± 1 58	± 2 00	± 1 49
S E.M	± 0 10	± 0 24	± 0 41	± 0 14
n	27	42	24	93
< 1 0 %	22 (81 %)	18 (43 %)	11 (46 %)	51 54 8 %
≧ 1 0 %	5 (19 %)	24 (57 %)	13 (54 %)	42 45 2 %

group I The difference when compared with groups II and III was highly significant ($P<0\,01$) but these last two groups did not differ significantly from one another

To appraise the degree of MHA poikilocytosis, the cases were divided into two groups One was made up of patients with MHA poikilocytosis below 1 0 per cent, the other of those with poikilocytosis 1 0 per cent or higher The results showed that the total study included 42 patients or 45 2 per cent with a value of 1 0 per cent or higher In the various groups, the distribution was in accordance with the mean values, as will be seen in Table 4 Exceptionally severe MHA poikilocytosis was demonstrated in 5 patients, the rate being in one of them 5 0 per cent and in the others higher Two of these patients belonged to group II and three to group III

The MHA poikilocytosis was not related to the patient s age ($r = -0\,08$)

Nor were there any differences between the sexes as regards MHA poikilocytosis The mean for men was $1\,51 \pm 1\,66$ per cent and for women $1\,31 \pm 1\,51$ per cent

MHA POIKILOCYTOSIS IN RELATION TO HEMOGLOBIN AND HEMATOCRIT VALUES

MHA poikilocytosis increased with an increase of severity of anemia The correlation between the hemoglobin concentration and MHA poikilocytosis in the total series was

$r = -0\,33$ ($P<0\,01$, $t = 3\,34$)

In groups II and III, in which MHA poikilocytosis was more frequent, the corresponding correlation was better in group II $r = -0\,41$ ($P<0\,01$, $t = 2\,86$), in group III $r = -0\,46$ ($P<0\,025$, $t = 2\,45$) No correlation was present in group I

The correlation between the hematocrit reading and MHA poikilocytosis obtained for the whole series was

$r = -0\,35$ ($P<0\,01$ $t = 3\,19$)

In group II the correlation was $r = -0\,48$ ($P<0\,01$, $t = 3\,17$), but in groups I and III there was no significant correlation

Table 5 shows the way in which MHA poikilocytosis was related to the grade of severity of the anemia in the whole series The mean MHA poikilocyte values rose as the anemia became more severe, corresponding to a negative correlation The means for the two most severe groups of anemia (hemoglobin –5 9 and 6 0–8 9 g/100 ml) differed highly significantly ($P<0\,01$, $t = 2\,80$) from the two groups with milder anemia (hemoglobin 9 0–11 4 and 11 5– g/100 ml) It is also apparent from the table that the number of patients with MHA poikilocytosis of

Table 5 *Mean (± S D) MHA poikilocyte counts and classification according to degree of poikilocytosis compared with hemoglobin levels*

Hemoglobin g/100 ml	MHA poikilocytosis %		
	Mean ± S D	< 1 0	≥ 1 0
– 5 9	3 90 ± 1 55	0	2 (100 %)
6 0 – 8 9	1 95 ± 1 93	15 (40 %)	23 (60 %)
9 0 – 11 4	0 93 ± 0 69	21 (60 %)	14 (40 %)
11 5 –	0 76 ± 1 29	15 (83 %)	3 (17 %)

1 0 per cent and over increased with greater severity of the anemia In conformity with this result, the mean hemoglobin level of patients with an MHA poikilocyte count below 1 0 per cent was higher (10 23 ± 2 08 g/100 ml) than in the rest of the patients (8 81 ± 1 66 g/100 ml) This difference was very highly significant ($P < 0\ 001$, $t = 3\ 58$)

The two patients with the most severe anemia had MHA poikilocytosis exceeding 1 0 per cent (2 8 per cent and 5 0 per cent) whereas only 3 patients with hemoglobin levels of 11 5 g/100 ml and over had a similar, rather high poikilocyte value All three of these were men with mild anemia their hemoglobin values being 11 5 11 7 and 12 6 g/100 ml The highest MHA poikilocytosis obtained for a nonanemic patient was 0 6 per cent This patient was a woman showing a hemoglobin content of 11 5 g/100 ml

MHA POIKILOCYTOSIS IN RELATION TO LEUCOCYTE COUNT AND TO PLATELET COUNT

No significant correlation could be demonstrated between the number of leucocytes and MHA poikilocytosis in the total series or in any of the groups

Neither was there any significant correlation between the platelet count and MHA poikilocytosis Study of the total material showed that patients with MHA poikilocytosis of 1 0 per cent or higher had a mean platelet count of 199080 ± 73270/mm³, *which was slightly lower* than the mean for the rest of the series 218090 ± 59470/mm³ The difference, however, was not statistically significant ($P < 0\ 30$)

LEUCOCYTES AND PLATELETS

In regard to the mean leucocyte counts the groups did not differ significantly from one another, as seen in Table 6 Five out of 89 subjects studied had leucocyte counts exceeding 15000/mm³ Of these 5, group III included 3 patients, and group I and II one each

The three groups did not differ as regards the number of platelets either These cells were counted in 68 cases, and in 18 additional patients the number of platelets in the smear was estimated to be normal In only one patient was the number of platelets lower than 100,000/mm³ This was a group II case

OSMOTIC RESISTANCE OF RED CELLS

The osmotic resistance of erythrocytes was tested in 80 patients Twenty four of these belonged to group I, 37 to group II, and 19 to group III Osmotic resistance was found to be normal in 36 patients or 45 0 per cent There were thus 44 patients with abnormal osmotic resistance These were distributed into

Table [illegible] *Leucocyte and platelet counts in various groups and in total series*

	Group I	Group II	Group III	Total
Leucocytes/mm³				
Mean	8 029 6	8 151 2	9 652 4	8 468 5
Range	3 900—25 200	3 700—19 900	4 700—23 300	3 700—25 200
S D	± 4 257 8	± 3 205 6	± 4 508 1	± 3 871 2
S E.M	± 819 4	± 500 6	± 983 7	± 410 3
n	27	41	21	89
Platelets/mm³				
Mean	203 470	214 930	201 230	208 300
Range	115 000—332 000	89 000—424 000	108 000—278 400	89 000—424 000
S D	± 65 420	± 73 120	± 59 350	± 67 840
S E.M	± 15 010	± 12 927	± 14 395	± 8 227
n	19	32	17	68

the various groups as follows 14 patients in group I (58 per cent), 20 in group II (54 per cent), and 10 in group III (54 per cent) In this respect there were no inter group differences

A comparison of the hemolysis curves in the group of abnormal resistance indicated that the divergences from the normal range were slight A slight increase in resistance was noted in 13 patients (30 per cent) This number included 5 patients of group I 4 of group II and 4 of group III Sixteen patients (36 per cent) showed a result according to which complete hemolysis was not attained until NaCl concentration decreased below 0 20 per cent Of these 16 two were group I cases, ten group II cases and four group III cases In 9 patients (20 per cent) hemolysis was found to begin at a time when NaCl concentration was higher than 0 50 per cent, the resistance being otherwise normal Among these nine there were six patients belonging to group I, one belonging to group II and two to group III A slightly reduced osmotic resistance was present in 6 patients (14 per cent) this number included one case of group I the other five being group II cases

ERYTHROCYTE LIFE SPAN

The erythrocyte lifetime was determined for 12 patients 4 belonging to group I, 5 to group II and 3 to group III The $T^1/_2Cr^{51}$ values recorded and the corresponding hemoglobin reticulocyte and serum creatinine values appear in Table 7

The life span of erythrocytes was reduced in 5 patients (one in group I 2 in group II and 2 in group III) In addition one patient of group I and another of group II showed an erythrocyte life span which was at the lowest normal limit The five patients with reduced erythrocyte life span included four who were definitely anemic and all five had serum creatinine levels exceeding 2 0 mg/100 ml

The erythrocyte life span could be correlated neither with hemoglobin nor with the serum creatinine concentration

SERUM IRON AND TOTAL IRON BINDING CAPACITY

The iron content of serum was estimated in 88 patients and total iron binding capacity (TIBC) in 82 The values con

cerned for the various groups and the total series are presented in Table 8

The serum iron content was normal (men 60–150 μg/100 ml, women 50–140 μg/100 ml) in 50 patients or 56 8 per cent of the total, diminished in 32 patients or 36 4 per cent, and increased in [illegible] patients or 6 8 per cent

The total iron binding capacity was normal in 31 patients of the total series or 37 8 per cent, reduced in 44 or 53 7

Table 7 *$T\frac{1}{2}Cr^{51}$ values and corresponding hemoglobin, reticulocyte and creatinine values in patients with recorded erythrocyte life span*

No	$T\frac{1}{2}Cr^{51}$ days	Hemoglobin g/100 ml	Reticulocytes %	Creatinine mg/100 ml
Group I				
1	17	7 8	9 8	2 3
2	25	8 2	3 1	1 2
3	30	8 8	0 5	2 5
4	36	10 0	8 9	0 9
Group II				
5	14	7 6	1 4	2 5
6	19	12 6	6 1	2 1
7	25	6 9	5 8	3 6
8	36	9 1	2 8	1 5
9	40	9 4	2 5	3 6
Group III				
10	22	8 8	4 5	2 4
11	22	8 7	9 0	6 3
12	33	10 2	5 3	10 2

Table 8 *Serum iron and total iron binding capacity (TIBC) values in various groups and in total series*

	Group I	Group II	Group III	Total
Serum iron μg/100 ml				
Mean	60 36	80 10	81 73	74 73
Range	10–165	14–230	17–132	10–230
S D	± 36 14	± 19 73	± 34 89	± 42 64
S E M	± 7 23	± 7 86	± 7 28	± 4 55
n	25	40	23	88
TIBC μg/100 ml				
Mean	258 40	254 48	269 43	259 73
Range	186–[illegible]	86–560	159–425	86–560
S D	± 75 18	± 91 51	± 68 45	± 81 39
S E M	± 16 03	± 15 05	± 14 27	± 8 99
n	22	37	23	82

per cent, and increased in 7 or 8.5 per cent.

The results in the various groups were as follows

Group I Serum iron was found to be reduced in 12 patients (48 per cent) out of 25 patients studied. Three of the 12 subjects were men. A normal serum iron level was obtained in 12 patients and an increased level in one. Staining of bone marrow for iron was carried out in eight of the 12 patients with reduced serum iron. The staining was positive in all eight cases. The remaining four showed the hemoglobin concentration in bone marrow erythroblasts to be good. In three of these four TIBC was normal, in one reduced.

TIBC was determined in 22 cases. It was decreased in 13, normal in 8 and increased in one patient.

Group II 14 out of 40 subjects studied (35 per cent) had a reduced serum iron content. Of these 14, four were men. Serum iron was normal in 21 patients and increased in 5. Staining of bone marrow for iron was carried out in five of the subjects with reduced serum iron values. Staining was positive in 3. Two others showed a normal TIBC and the hemoglobin concentration in the erythroblasts showed no deficiency. TIBC was determined in eight of the remaining nine: it was reduced in 2, normal in 3 and increased in 3. The hemoglobin content of all erythroblasts with increased TIBC was good. In one case the TIBC value was not determined: this patient's bone marrow was nearly aplastic.

TIBC was determined in 37 patients. It was diminished in 21, normal in 13, and elevated in 3.

Group III Six of the 23 subjects studied (26 per cent) showed reduced serum iron. Of these, three were men. The serum iron value obtained for the remaining 17 was normal. Staining of bone marrow for iron was performed in three of the six patients having lowered serum iron levels: the staining was positive in two of them. In the case of all six of these patients the hemoglobin content of the erythroblasts was good.

TIBC was tested in 23 patients. It was reduced in 10, normal in 10 and elevated in 3 cases.

Comparison of the mean iron value in the various groups showed that it was lower in group I than in groups II and III, which showed approximately the same average value. The difference was not statistically significant, however. Combination of groups II and III resulted in the mean 80.44 ± 44.58 μg/100 ml. This was significantly higher ($P < 0.05$, $t = 2.00$) than the mean for group I.

The mean total iron binding capacity values did not differ significantly from one group to another.

HAPTOGLOBIN

The haptoglobin levels were determined in 28 patients. Thirteen of these belonged to group I, 10 to group II and 5 to group III. The values in the three groups and the total series are seen in Table 9.

Table 9 *Serum haptoglobin values (g/l) in various groups and in total series*

	Group I	Group II	Group III	Total
Mean	1.59	1.18	1.38	1.41
Range	0.01–3.89	0.01–2.08	0.04–2.83	0.01–3.89
S.D.	± 1.39	± 0.70	± 1.09	± 1.14
S.E.M.	± 0.37	± 0.22	± 0.49	± 0.22
n	13	10	5	28

Comparison revealed no significant differences between the groups Eight patients were found to have low haptoglobin levels (below 0 3 g/l) In five of these the low haptoglobin concentration was verified by immunoelectrophoresis Low values were recorded for five patients in group I, two in group II, and one in group III Increased bone marrow erythropoiesis pointing to hemolysis was observed in seven of the 8 patients and increased reticulocyte counts in 4

Elevated haptoglobin concentrations (>1 9 g/l) were found in 9 patients Six of these were group I cases, one belonged to group II and two to group III

The haptoglobin value was related to neither hemoglobin ($r = -0\ 12$) nor serum creatinine ($= +0\ 12$) A correlation was present mainly with the reticulocyte count ($r = -0\ 35$), but it was not statistically significant ($0\ 10 > P > 0\ 05$)

ANTIGLOBULIN ANTIBODIES (COOMBS)

Eighty of the patients were examined for antiglobulin antibodies by means of the Coombs test Of these patients, 24 were in group I, 39 in group II, and 17 in group II

All tests were negative for antibodies

SERUM CREATININE IN RELATION TO HEMOGLOBIN AND HEMATOCRIT VALUES AND RETICULOCYTE AND MHA POIKILOCYTE COUNTS

HEMOGLOBIN AND HEMATOCRIT VALUES

Using serum creatinine as a measure of renal insufficiency the results showed that the anemia increased with an increase in the severity of the renal insufficiency The correlation between serum creatinine and hemoglobin concentration for the whole series was

$r = -0\ 43$ ($P<0\ 001$, $t = 4\ 48$)

The correlations in the three groups were as follows

Group I $r = -0\ 32$ ($P>0\ 05$)
Group II $r = -0\ 45$ ($P<0\ 01$, $t = 3\ 17$)
Group III $r = -0\ 64$ ($P<0\ 001$, $t=3\ 89$)

Thus, a significant correlation was found to exist between serum creatinine and hemoglobin both in the whole study and in the groups associated with ingestion of phenacetin containing drugs

Eight of the 12 nonanemic patients had increased levels of serum creatinine All patients whose serum creatinine values were 4 5 mg/100 ml or in excess of it were anemic

The relation between serum creatinine and the hematocrit reading was similar to the above In the total series the correlation was

$r = -0\ 49$ ($P<0\ 001$, $t = 4\ 48$)

For the three groups the relations were as follows

Group I $r = -0\ 40$ ($P>0\ 05$)
Group II $r = -0\ 58$ ($P<0\ 001$, $t=4\ 19$)
Group III $r = -0\ 56$ ($P<0\ 05$ $t = 2\ 80$)

In studying the way in which the anemia was related to the severity of the renal insufficiency in the various groups, the mean hemoglobin value was compared with the mean creatinine level in the respective group The result appears in Table 10 The renal disease was on an

Table 10 *Mean (± S D) hemoglobin concentration compared with mean (± S D) serum creatinine in the various groups*

Group	Hemoglobin g/100 ml	Serum creatinine mg/100 ml
I	9 38 ± 1 41	3 54 ± 2 82
II	9 39 ± 2 26	4 05 ± 3 46
III	10 17 ± 2 11	5.54 ± 3 80

average of slightest degree in group I but the hemoglobin value was lowest Group III was the least anemic one, but the renal insufficiency in this group was more severe than in the others From this it is seen that a renal insufficiency of equal severity caused the highest degree of anemia in group I and the mildest anemia in group III Statistically, however, the differences were not significant (0 10 >P>0 05)

RETICULOCYTES

The severity of the renal insufficiency did not bear a definite relationship to reticulocytosis The correlation between serum creatinine and reticulocytosis was significant neither in the whole series (r = +0 20) nor in any of the groups

There was considerable reticulocytosis even in severe renal insufficiency Of 13 patients whose serum creatinine levels ranged over 8 0 mg/100 ml, nine were found to have a reticulocytosis exceeding 3 0 per cent

MHA POIKILOCYTES

MHA poikilocytosis increased slightly with increasing severity of the renal disease There was however, only a weak correlation between serum creatinine and MHA poikilocytosis (r = +0 26 P<0 05 t = 2 60) Study of each of the groups separately revealed a significant correlation in none of them

Comparison of the MHA poikilocytosis as classified in the groups with the serum creatinine level showed that the patients in whom the former value was 1 0 per cent or more had a serum creatinine mean of 5 34 ± 4 04 mg/100 ml which was highly significantly (P<0 01 t = 2 79) greater than the corresponding mean 3 42 ± 2 56 mg 100 ml for those patients whose MHA poikilocytosis was lower than 1 0 per cent

EFFECT OF THE DURATION AND DISCONTINUANCE OF ANALGESIC ABUSE ON THE SERUM CREATININE AND HEMOGLOBIN AND ON THE RETICULOCYTE, MHA POIKILOCYTE, LEUCOCYTE AND PLATELET COUNTS

SERUM CREATININE AND HEMOGLOBIN

The effect exercised on the serum creatinine and hemoglobin values by the duration of analgesic abuse and of its discontinuance is presented in Table 11

As regards serum creatinine it was found that those patients who had used analgesics up to the time of admission to hospital had a significantly higher mean serum creatinine content than those who had already stopped taking analgesics In dividual cases were not followed for changes in kidney function after abuse stopped so it is not possible to say on the basis of the results whether its discontinuance affects this function favourably The mean serum creatinine value did not rise as duration of the abuse increased the lowest mean was obtained in the group in which abuse had lasted over 10 years There were no significant differences however

Anemia was on an average somewhat milder in patients who had stopped taking analgesics than in those who had used them until admitted to hospital The effects of discontinued abuse was not studied in individual cases The anemia was not aggravated with increasing duration of abuse the mean hemoglobin value was highest in the group in which abuse had continued for over ten years However no differences of any significance were found

RETICULOCYTES AND MHA POIKILOCYTES

Table 12 illustrates the effect exerted on the reticulocyte and MHA poikilocyte counts by the duration of phenacetin abuse and its discontinuance

Table 11 *Effect of duration and discontinuance of analgesic abuse on serum creatinine and hemoglobin values*

	Ingestion of analgesics				
	Stopped	Continued	Duration of abuse, years		
			< 3	3–10	> 10
Serum creatinine mg/100 ml					
Mean	3.17	5.21*	5.42	4.78	3.63
Range	0.6–8.2	0.6–16.5	1.2–12.4	0.6–16.5	0.8–10.2
S.D.	± 2.19	± 3.96	± 4.00	± 3.77	± 3.00
S.E.M.	± 0.49	± 0.59	± 0.42	± 0.58	± 0.77
[illegible]	20	46	8	43	15
Hemoglobin g/100 ml					
Mean	10.17	9.46	9.51	9.52	10.21
Range	6.3–13.2	5.5–15.2	6.2–12.1	5.5–15.2	5.5–14.4
S.D.	± 2.25	± 2.30	± 2.24	± 2.22	± 2.31
S.E.M.	± 0.50	± 0.32	± 0.79	± 0.34	± 0.60
n	20	46	8	43	15

* difference statistically significant ($P < 0.05$, $t = 2.16$)

Table 12 *Effect of duration and discontinuance of analgesic abuse on reticulocyte and MHA poikilocyte counts*

	Ingestion of analgesics				
	Stopped	*Continued*	Duration of abuse, years		
			< 3	3–10	> 10
Reticulocytes (%)					
Mean	2.76	3.64	2.43	3.64	3.10
Range	0.4–6.1	0.7–14.4	1.0–5.5	0.4–13.4	0.8–11.0
S.D.	± 2.90	± 2.85	± 1.54	± 3.11	± 2.76
S.E.M.	± 0.65	± 0.44	± 0.55	± 0.49	± 0.71
n	20	[illegible]	8	40	15
MHA poikilocytes (%)					
Mean	1.28	1.86	1.23	1.72	1.83
Range	0–3.5	0.1–7.5	0.1–4.8	0–5.7	0.1–7.5
S.D.	± 1.14	± 1.91	± 1.52	± 1.48	± 2.45
S.E.M.	± 0.26	± 0.28	± 0.54	± 0.23	± 0.67
n	20	46	8	43	15

The mean reticulocyte counts did not differ significantly in relation to the duration of abuse Results indicated that reticulocytosis was on an average increased both in those who had already stopped using analgesics and in those who still used them when admitted to hospital Nine out of 20 who had stopped taking the drugs had a reticulocytosis exceeding 2 0 per cent, and seven of these 9 had a reticulocytosis of 3 0 per cent and over The highest value was 13 4 per cent Of these 9, eight had not taken phenacetin-containing analgesics for over two years and one for one year

The mean MHA poikilocyte count rose to some extent with increasing duration of the abuse The differences, however, were only slight Stopping of the abuse did not significantly affect the occurrence of MHA poikilocytosis either, though those still taking the drugs on admission to hospital showed slightly higher mean value Of 20 patients who had stopped taking analgesics, ten had MHA poikilocyte values of 1 0 per cent and over Nine of these ten had not used these drugs for over two years one not for a few months On the other hand 9 patients who had continued the abuse until admitted to hospital nevertheless had only slight poikilocytosis (0 1—0 4 per cent) All of these had been using phenacetin-containing drugs daily up to the time of admission four of them for over ten years In three of the nine patients measurement of serum creatinine yielded normal values

LEUCOCYTES AND PLATELETS

The leucocyte and platelet counts were not affected by the duration of abuse as will be seen from Table 13 Those who had left off using analgesics had a significantly lower leucocyte count that those who had continued the abuse No differences were detected in platelet counts

Table 13 *Effect of duration and discontinuance of analgesic abuse on leucocyte and platelet counts*

	Ingestion of analgesics				
	Stopped	Continued	Duration of abuse, years		
			< 3	3—10	> 10
Leucocytes/mm³					
Mean	7 189 0	9 309 3*	8 600 0	8 205 1	9 873 3
Range	3 700—12 900	4 300—23 300	6 800—12 500	3 700—19 900	5 100—23 300
S D	± 2 368 1	± 4 049 2	± 2 379 7	± 3 515 7	± 4 700 1
S E.M.	± 543 3	± 617.5	± 814 3	± 563 0	± 1 213 6
n	19	43	8	39	15
Platelets/mm³					
Mean	215 710	207 950	195 280	216 500	200 400
Range	125 000— 375 000	89 000— 424 000	89 000— 272 000	115 000— 424 000	108 000— 272 000
S D	± 68 540	± 69 140	± 63 360	± 73 270	± 57 650
S E.M	± 18 319	± 11 687	± 23 951	± 12 952	± 18 231
n	14	35	7	32	10

* difference statistically significant (P < 0 05 t = 2 12)

CELLULARITY AND ERYTHROPOIESIS

Tables 14 and 15 classify the patients according to degree of cellularity and marrow erythropoiesis

A rise in cellularity was more frequent in groups I and II but there were no statistically significant differences between the groups Cellularity was increased in 23 6 per cent of the total number, affecting in 18 cases of 22 both the myeloid and erythroid cells, in the rest, the myeloid-erythroid ratio was high In 17 patients or 18 3 per cent, the cellularity of the bone marrow was diminished, this number including only one case with aplastic bone marrow, however This was a group II case

As regards erythropoiesis, the groups did not differ from one another Erythropoiesis was increased in over 40 per cent of the patients in all groups Erythropoietic reduction was found in 14 0 per cent The erythropoiesis was normoblastic in most cases Eight patients showed a slightly macroblastic bone marrow, the rate of erythropoiesis being increased in five of these

Table 14 *Classification according to bone marrow cellularity*

Cellularity	Group I	Group II	Group III	Total
Reduced	5	7	5	17
	(18 %)	(17 %)	(21 %)	18 3 %
Normal	15	22	17	54
	(56 %)	(52 %)	(71 %)	58 1 %
Increased	7	13	2	22
	(26 %)	(31 %)	(8 %)	23 6 %

Table 15 *Classification according to bone marrow erythropoiesis*

Erythropoiesis	Group I	Group II	Group III	Total
Reduced	3	8	2	13
	(11 %)	(19 %)	(8 %)	14 0 %
Normal	12	17	12	41
	(44 %)	(41 %)	(50 %)	44 1 %
Increased	12	17	10	39
	(44 %)	(41 %)	(42 %)	41.9 %

RELATION OF CELLULARITY AND OF ERYTHROPOIESIS TO SERUM CREATININE HEMOGLOBIN AND RETICULOCYTE VALUES

In Tables 16 and 17 are presented the mean serum creatinine and hemoglobin values and mean reticulocyte counts as compared with cellularity and erythropoiesis.

Both cellularity and erythropoiesis were found to rise with greater severity of the renal insufficiency. This appeared more clearly in the case of erythropoiesis. The differences were not, however, significant. Thirteen patients showed decrease of erythropoiesis, in five of these serum creatinine was within normal range and in only 3 over 7.0 mg/100 ml. An increase in erythropoiesis was noted in 39 patients, only three of these had normal serum creatinine, 7 showing a value of 10.0 mg/100 ml and over.

In studying hemoglobin it was found that both cellularity and erythropoiesis increased as the anemia became more severe. The difference was not significant as regards cellularity. However, it was observed that the group with increased erythropoiesis had a significantly ($P<0.05$, $t = 1.99$) lower mean hemoglobin value as compared to the group with reduced erythropoiesis and a highly significantly ($P<0.01$, $t = 3.06$) lower mean hemoglobin as compared to the group with normal erythropoiesis.

It is clear that a rise in erythropoiesis is also reflected in the reticulocyte percentage. This was distinctly revealed by a comparison of mean reticulocyte counts with cellularity and with erythropoiesis. There was a highly significant ($P<0.01$) increase in mean reticulocyte count as compared to the patients with normal or reduced cellularity. A more distinct

Table 16. *Mean (± S.D.) serum creatinine, hemoglobin and reticulocyte values in relation to cellularity of bone marrow*

Cellularity	Serum creatinine mg/100 ml	Hemoglobin g/100 ml	Reticulocytes %
Reduced	3.54 ± 3.07	9.99 ± 2.40	2.19 ± 2.38
Normal	4.38 ± 3.33	9.70 ± 2.03	3.08 ± 2.38
Increased	4.64 ± 3.98	9.01 ± 1.60	4.92 ± 3.50

Table 17. *Mean (± S.D.) serum creatinine, hemoglobin and reticulocyte values in relation to erythropoiesis of bone marrow*

Erythropoiesis	Serum creatinine mg/100 ml	Hemoglobin g/100 ml	Reticulocytes %
Reduced	3.43 ± 2.89	10.06 ± 2.70	1.84 ± 2.50
Normal	3.78 ± 3.15	10.16 ± 1.91	2.08 ± 1.27
Increased	5.11 ± 3.77	8.83 ± 1.64	5.14 ± 3.10

difference was observed when comparing reticulocytosis with erythropoiesis. When erythropoiesis was increased, the mean reticulocyte count was very highly significantly ($P<0.001$) greater than in the case of normal or reduced erythropoiesis.

EFFECT OF DURATION AND DISCONTINUANCE OF ANALGESIC ABUSE ON BONE MARROW CELLULARITY AND ERYTHROPOIESIS

Tables 18 and 19 illustrate the way in which the cellularity of bone marrow and erythropoiesis are affected by the duration of abuse and by its cessation.

Only slight differences were found in these respects between the groups based on duration of abuse.

Comparison of those who had already stopped the drugs with those *still taking* them at the time of admission to hospital did not reveal any significant differences either. A group of 20 who had stopped *taking analgesics included 8* with increased erythropoiesis. Seven of these 8 had not used phenacetin-containing drugs for over two years and one for two months.

Table 18 *Effect of duration and discontinuance of analgesic abuse on cellularity of bone marrow*

Cellularity	Ingestion of analgesics				
	Stopped	Continued	Duration of abuse, years		
			< 3	3–10	> 10
Reduced	1 (20 %)	8 (17 %)	3 (37.5 %)	7 (16 %)	2 (13 %)
Normal	8 (40 %)	31 (67 %)	5 (62.5 %)	24 (56 %)	10 (67 %)
Increased	[illegible] (40 %)	7 (15 %)	0	12 (28 %)	3 (20 %)

Table 19 *Effect of duration and discontinuance of analgesic abuse on erythropoiesis of bone marrow*

Erythropoiesis	Ingestion of analgesics				
	Stopped	Continued	Duration of abuse, years		
			< 3	3–10	> 10
Reduced	2 (10 %)	8 (17 %)	1 (12.5 %)	5 (12 %)	4 (27 %)
Normal	10 (50 %)	19 (41 %)	4 (50 %)	19 (44 %)	6 (40 %)
Increased	8 (40 %)	19 (41 %)	3 (37.5 %)	19 (44 %)	5 (33 %)

MHA POIKILOCYTOSIS AS COMPARED WITH OTHER FINDINGS INDICATIVE OF INCREASED HEMOLYSIS

RETICULOCYTOSIS

The mean MHA poikilocyte counts at various levels of reticulocytosis are shown in Table 20.

The means rose as the reticulocytosis increased. Patients with a reticulocytosis of over 3.0 per cent had a mean MHA poikilocyte count of 2.06±2.00 per cent — a figure significantly ($P<0.05$, $t = 2.08$) greater than obtained for those with a reticulocytosis ranging from 2.1 to 3.0 per cent and highly significantly ($P<0.01$, $t = 3.30$) greater than for those with normal reticulocytosis.

A significant correlation ($r = +0.33$, $P<0.01$, $t = 3.25$) was found to exist between reticulocytes and MHA poikilocytes.

Table 20 and Figure 5 classify the patients by degree of reticulocytosis as compared with the degree of MHA poikilocytosis.

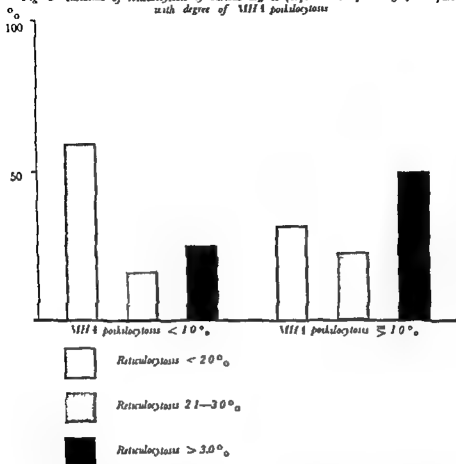
Similarly as in the case of the means, the number of patients with MHA poikilocytosis of 1.0 per cent or higher increased significantly ($P<0.05$, $\chi^2 = 8.46$) with a rise in reticulocytosis. The mean reticulocyte counts in patients having MHA poikilocyte values of 1.0 per cent and over was 4.45±3.29 per cent and the mean in patients with a poikilocytosis below 1.0 per cent was 2.47±1.99 per cent. The difference was very highly significant ($P<0.001$, $t = 3.52$).

Of the patients with MHA poikilocyte values of 1.0 per cent and over, 48.7 per cent showed a reticulocyte value exceeding 3.0 per cent, 22.0 per cent a value ranging from 2.1 to 3.0 per cent and 31.3 per cent a value below 2.0 per cent. In the group with MHA poikilocytosis below 1.0 per cent these percentages were respectively 24.5, 16.4 and 59.1.

Table 20. *Mean (± S.D.) MHA poikilocyte counts and classification according to degree of poikilocytosis compared with reticulocytosis*

Reticulocytosis %	MHA poikilocytosis %		
	Mean ± S.D.	< 1.0	≧1.0
–2.0	0.91 ± 1.12	29 (71 %)	12 (29 %)
2.1–3.0	1.14 ± 0.96	8 (47 %)	9 (53 %)
3.1–	2.06 ± 2.00	12 (37.5 %)	20 (62.5 %)

Fig 5 Incidence of reticulocytosis of various degrees (expressed as percentages) compared with degree of MHA poikilocytosis



BONE MARROW ERYTHROPOIESIS

Table 21 lists the mean MHA poikilocyte counts as compared with the rate of marrow erythropoiesis

There was a significant correlation between MHA poikilocytosis and increased erythropoietic activity In the group with increased erythropoiesis, the mean MHA poikilocyte count was $2·03 \pm 1·87$ per cent — a figure significantly ($P<0·02$, $t = 2·48$) higher than the corresponding mean obtained in the group with reduced erythropoiesis and highly significantly ($P<0·01$, $t = 3·39$) greater than in the group with normal erythropoiesis

The degree of erythropoiesis in relation to MHA poikilocytosis is illustrated in Table 21 and Figure [illegible] As erythropoiesis became progressively greater, the number of cases with an MHA poikilocytosis of or exceeding 1·0 per cent increased highly significantly ($P<0·01$, $\chi^2 = 12·84$)

Of the patients with MHA poikilocytosis of 1·0 per cent and over, 61·9 per cent were found to have increased erythropoiesis, 31·0 per cent normal, and 7·1 per cent reduced erythropoiesis The same percentages in the group with MHA poikilocytosis below 1·0 per cent were 25·5, 54·9 and 19·6 respectively

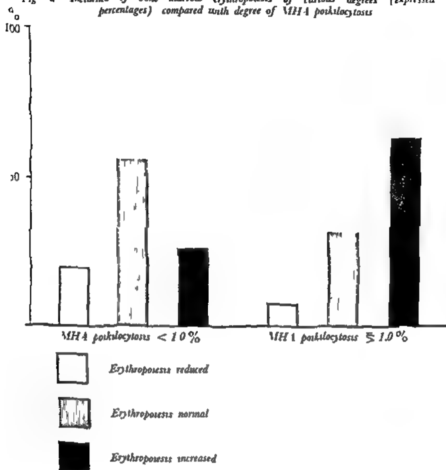


Fig 6 Incidence of bone marrow erythropoiesis of various degrees (expressed as percentages) compared with degree of MHA poikilocytosis

Table 21 *Mean (± S D) MHA poikilocyte counts and classification according to degree of poikilocytosis compared with bone marrow erythropoiesis*

Erythropoiesis	MHA poikilocytosis %		
	Mean ± S D	< 1 0	≧ 1 0
Reduced	0 86 ± 1 34	10 (77 %)	3 (23 %)
Normal	0 92 ± 0 99	28 (68 %)	13 (32 %)
Increased	2 03 ± 1 87	13 (33 %)	26 (67 %)

OSMOTIC RESISTANCE OF RED CELLS

No interdependence was found between MHA poikilocytosis and the osmotic resistance of erythrocytes

In the group with normal osmotic resistance, the mean MHA poikilocyte count was 1 07±1 49 per cent As already stated, there were 13 patients showing increased osmotic resistance of the red cells The mean poikilocyte count recorded for these was 1 43±1 48 per cent In the case of 16 patients, the red cells were not completely hemolysed until the NaCl concentration dropped below 0 20 per cent In this group the mean MHA poikilocytosis was 1 61±1 87 per cent In 9 patients in whom red cell hemolysis started when NaCl concentration was higher than 0 50 per cent, being otherwise normal, the corresponding mean was 1 48±1 93 per cent, in 6 patients with slightly decreased osmotic resistance it was 1 15±0 76 per cent In respect of MHA poikilocytosis the diverging groups differed neither from those with normal osmotic resistance nor from one another

ERYTHROCYTE LIFE SPAN

Red cell survival time was only determined in 12 patients and consequently no definite conclusions can be drawn as to the relationship between MHA poikilocytosis and erythrocyte life span No significant correlation could be found in this respect However, four of the 5 patients with reduced red cell survival did show high MHA poikilocyte values of over 2 8 per cent Of the seven having normal red cell lifetimes only two had a similar high poikilocyte value

Nine of the patients thus studied had MHA poikilocytosis of 1 0 per cent and over The mean $T^{1}/_{2}Cr^{51}$ calculated for these patients was 26 44±7 76 days Those whose MHA poikilocyte value was below 1 0 per cent had a slightly higher mean, 27 33±11 67 days

SERUM IRON

Comparison of serum iron and MHA poikilocytosis showed no correlation of any significance

On the basis of the MHA poikilocytosis classification here used, it was found that, in the group with MHA poikilocytosis of 1 0 per cent and over, the mean serum iron concentration was 87 33±47 43 μg/100 ml This was highly significantly ($P<0\ 01$, $t=2\ 71$) in excess of the corresponding mean, 63 23±35 54 μg/100 ml, in the group in which MHA poikilocytosis was below 1 0 per cent

HAPTOGLOBIN

Comparison of the haptoglobin concentration and MHA poikilocytosis revealed no significant correlation It was stated in a previous section that low haptoglobin concentrations were found in 8 patients In three of these MHA poikilocytosis was above 1 0 per cent

While in the group with MHA poikilocytosis of 1 0 per cent and over (12 patients) the mean haptoglobin concentration was 1 09±0 79 g/l, the corresponding mean in the group with a poikilocytosis below 1 0 per cent (16 patients) was 1 64±1 27 g/l The difference was not statistically significant

SERUM BILIRUBIN

Serum bilirubin was measured in 85 patients In 2, the bilirubin value was over 1 0 mg/100 ml (1 1 and 1 2 mg/100 ml), in all others below it

No significant correlation could be established between serum bilirubin concentration and MHA poikilocytosis However, in the group showing MHA poikilocytosis of 1 0 per cent and over, the mean bilirubin level was 0 48±0 26 mg/100 ml, thus significantly ($P<0\ 05$, $t = 2\ 02$) higher than the corresponding mean, 0 38±0 17 mg/100 ml, for the group in which poikilocytosis was below 1 0 per cent

DISCUSSION

BLOOD AND BONE MARROW

Despite the fact that anemia is often present in very early cases of renal insufficiency, it seldom reaches a severe stage even in far advanced kidney disease. In the present study the hemoglobin level was below 8.0 g/100 ml in 20 patients, representing 24.1 per cent of anemic cases. Indeed anemia has been found not to be aggravated beyond a certain level (Effersøe 1958, Roscoe 1952, Kasanen and Kalliomäki 1957). In conformity with previous researches the anemia was normochromic in most cases, but there were some with slight hypochromia or hyperchromia. There were no inter-group differences in this respect.

The anemia was progressively aggravated with increased severity of the renal insufficiency. Earlier studies have revealed a correlation between hemoglobin and serum creatinine concentration. In this study the corresponding correlation obtained for the total series was fairly good (r = −0.43). This is consistent with previous results (Kasanen 1958, Bock *et al.* 1962). Study of the groups separately revealed a significant correlation in those with accompanying abuse of phenacetin-containing analgesics; the best correlation was obtained in the group in which there were no chronic urinary tract infections.

Comparison of the mean hemoglobin and mean serum creatinine concentration in the various groups indicated that, in relation to the degree of renal insufficiency, the severest anemia occurred in the group with chronic urinary infection but without abuse of analgesics; the differences, however, were not significant. The opposite result would have been a more likely one since phenacetin is known to increase hemolysis. Differences in regard to degree of anemia have also been demonstrated in other kidney diseases. Bock and his co-workers (1962) reported that the same degree of anemia was attained at a lower creatinine level in chronic glomerulonephritis than in chronic pyelonephritis. These observations lend support to the view that there are obviously several concurrent factors involved in the mechanism of the anemia associated with chronic renal insufficiency and that the anemia is not directly related to the effect of retained substances. Above a definite degree of retention, however, the anemia is always recognizable (e.g. Bock and Thedering 1952, Kasanen and Kalliomäki 1957). The patients here studied were all anemic if their serum creatinine concentration was 4.5 mg/100 ml or over.

In the anemia of kidney disease, disturbances occur in bone marrow erythropoiesis as demonstrated in a great number of ferrokinetic studies (Finch *et al.* 1949, Joske *et al.* 1956, Desforges and Dawson 1958, Loge *et al.* 1958, Naets *et al.* 1960, Ragen *et al.* 1960, Esbach *et al.* 1967, Tagasuki and Imura 1967). This is not, however, evidenced as a reduction in the number of erythropoietic cells in the bone marrow. In this study a quantitative depression of erythropoiesis was found in 14.0 per cent of the patients, and it was not related to the serum creatinine value. Callen and Limarzi (1950) only encountered depressed erythropoiesis in cases with a very high

blood non protein nitrogen value (over 150 mg/100 ml) The cellularity of bone marrow and the degree of erythropoiesis showed agreement with previous reports (Andereggen 1946, Callen and Limarzi 1950), and the results did not differ from one group to another

The main emphasis in this work was on studying the part played by hemolysis in the anemia in the various groups

The reticulocyte count was increased in 54 4 per cent of the patients The data on reticulocytosis in renal anemia are variable, but in several reports some of the patients or even all have presented increased reticulocyte values (Bock and Thedering 1952, Gormsen and Gjorup 1955, Loge *et al* 1958, Shaw and Scholes 1967) There was a rise in reticulocytosis as anemia increased This was also observed by Shaw and Scholes (1967) Previous investigators (Friis *et al* 1959, 1960) reported a higher degree of reticulocytosis in renal patients who were phenacetin addicts than in patients whose renal insufficiency was not associated with phenacetin addiction The results obtained in the present study were not along the same lines

Bone marrow erythropoiesis was increased in 41 9 per cent of the total number of patients, and to the same extent in each group As anemia increased in severity, the rate of erythropoiesis also increased, in the same way as reticulocytosis Erythropoiesis increased with the severity of the renal insufficiency, but not to a significant extent Using only reticulocytosis and increased bone marrow erythropoiesis as criteria of hemolysis, almost half of the patients were found to have increased hemolysis In this respect the patients who had used analgesics did not differ from those who had not taken such drugs

Red cell survival time was determined in only a small proportion of the patients However, a shortening of red cell life span indicating increased hemolysis was observed in all groups, which result is in line with the high reticulocyte count and increased erythropoiesis The result differs from those of Nissen and Friis (1962) They found, in addition, that erythrocyte life span was only reduced in phenacetin abusers if phenacetin administration was continued during the study The patients here dealt with did not receive phenacetin containing analgesics while the study was in progress Erythrocyte lifetime was related neither to anemia nor to serum creatinine concentration This latter finding was also reported by Stewart (1967)

The osmotic resistance of the erythrocytes was normal in about half of the patients studied The divergences here noted were slight in agreement with earlier reports (Loge *et al* 1958, Desforges and Dawson 1958) There were no differences between the groups

The haptoglobin concentration was measured in part of the patients Intergroup differences were not detected From the point of view of increased hemolysis those cases are of interest in which the haptoglobin concentration was low Seven of the 8 patients concerned showed increased bone marrow erythropoiesis and 4 increased reticulocyte counts

Autoimmune hemolytic anemia was not present The same applies to a great number of previous investigations (Joske *et al* 1956 Kaye 1958 Loge *et al* 1958 Verel *et al* 1959)

MICROANGIOPATHIC HEMOLYTIC ANEMIA

The poikilocytosis characterizing microangiopathic hemolytic anemia is readily recognizable On close scrutiny of a blood smear it is possible to find even in a healthy individual red cells fulfilling the criteria for MHA poikilocytosis but the number of such cells is very small According to the report of Schwartz and Motto (1949) who studied 100 patients,

using these drugs The results do not permit any conclusions as to whether the discontinuance of abuse had a favourable effect on kidney function in individual cases Results pointing in this direction have been published (e g Schreiner 1965, Kasanen 1967) but it must be kept in mind that many of the patients concerned were under active treatment

The anemia did not increase in severity with the duration of analgesic abuse On the contrary, those patients who had used analgesics for over ten years had the mildest anemia The differences in this respect were not significant This result is in harmony with the serum creatinine values though, it is true, the correlation between hemoglobin and serum creatinine was not very good in the case of analgesic abusers either — as already stated above The patients who had left off using analgesics had anemia of somewhat milder degree than those who were still using analgesics when admitted to hospital The difference was not significant Bengtsson and Hood (1965) stated that the mean hemoglobin levels of renal patients rose as the duration and amount of abuse decreased This would seem to indicate that exactly phenacetin is of great importance in the development of anemia in patients with renal disease accompanied by analgesic abuse In this study, phenacetin was not found to increase the degree of anemia the anemia as such accompanied renal insufficiency Increased duration of abuse caused no significant change in reticulocytosis Those who had stopped using analgesics also had on an average higher reticulocyte counts than normal

In complete agreement with the above results, significant changes occurred neither in the cellularity of bone marrow nor in the degree of erythropoiesis with increased duration of analgesic abuse An increased erythropoietic activity arguing in favour of hemolysis occurred equally in those who had given up abuse and in those who continued abuse until they presented themselves for treatment

SUMMARY

The study was based on 93 patients suffering from insufficiency of the kidneys, divided into three groups as follows 1 those with chronic pyelonephritis (27 cases), 2 those with chronic pyelonephritis associated with regular abuse of phenacetin-containing analgesics (42 cases), and 3 those who had taken phenacetin containing drugs regularly but in whom chronic urinary infection had not been diagnosed (24 cases) The work was carried out for the purpose of studying possible differences between the above groups as regards the mechanism of anemia Special interest was devoted to the occurrence of a poikilocytosis typical of microangiopathic hemolytic anemia

There were no inter group differences as far as the degree of severity of anemia was concerned The anemia was normochromic in most cases

The reticulocyte counts were higher than normal in all groups but there were no significant differences between the groups Reticulocytosis was over 2 0 per cent in 54 4 per cent of the total series and over 3 0 per cent in 35 5 per cent

Leucocyte and platelet counts were on an average normal in the various groups

Osmotic resistance of red cells differed from normal in 5[illegible] 0 per cent of those studied (80 patients), yet the divergences were slight and no uniform change was observed The groups did not differ from one another

Erythrocyte life span was determined in Cr^{51} labelled red cells in 12 patients Shortening of red cell survival occurred in all the groups

Serum iron concentration was on an average reduced in all groups, most definitely in patients who had not used phenacetin containing analgesics, but the differences were not significant

Haptoglobin concentration did not differ from one group to another in the series studied (28 patients)

The Coombs test failed to reveal antiglobulin antibodies in any one of those examined (80 patients)

The hemoglobin and serum creatinine values proved to be related in the total series (r = — 0 43) This correlation varied in the different groups the best correlation occurring in patients who had used phenacetin containing analgesics but had no urinary tract infections (r = — 0 64) The correlation between hematocrit reading and serum creatinine was consistent with the above No correlation could be found between reticulocytosis and serum creatinine

Neither the duration nor the discontinuance of analgesic abuse significantly affected the hemoglobin concentration or the reticulocytosis The mean serum creatinine was significantly higher in patients who had continued using analgesics up to hospital admission than in those who had already stopped taking the drugs The duration of abuse had no significant effect on the serum creatinine value

The cellularity of bone marrow was reduced in 1[illegible] 3 per cent of the total number of patients, normal in 5[illegible] 1 per cent and increased in 23 6 per cent Intergroup differences were not significant Erythropoietic activity was reduced in 14 0 per cent of all patients, normal in

44 1 per cent, and increased in 41 9 per cent The groups did not differ significantly from one another

Both bone marrow cellularity and the degree of erythropoiesis increased with a rise in serum creatinine, but the change was not statistically significant Increased severity of anemia was associated with an increase in both bone marrow cellularity and in erythropoiesis, the latter only being significant

Neither duration nor discontinuance of analgesic abuse were found to play a significant part in bone marrow cellularity or in the rate of erythropoiesis

The poikilocytosis typical of microangiopathic hemolytic anemia (MHA poikilocytosis) was estimated quantitatively by balanced sampling In evaluating the results, the patients were divided into two groups one consisting of those showing an MHA poikilocytosis of 1 0 per cent or over and the other of those with a poikilocytosis below 1 0 per cent

The mean MHA poikilocyte count for the whole series was 1 38 per cent, and MHA poikilocytosis of 1 0 per cent or over occurred in 45 2 per cent of the patients Poikilocytosis was significantly more frequent in the groups of patients who had [illegible] of phenacetin containing analgesics In [illegible] groups however the mean was signifi[illegible] higher than the figure obtained [illegible] with blood donors serving as controls

The incidence of MHA poikilocytosis did not differ from one sex to the other and was not related to age

MHA poikilocytosis could be correlated to hemoglobin (r = − 0 33) and to hematocrit reading (r = − 0 35) A poikilocytosis of and exceeding 1 0 per cent was only encountered in anemic patients

Thrombocytopenia did not occur to any significant extent in association with MHA poikilocytosis

The correlation between MHA poikilocytosis and serum creatinine was weak (r = + 0 26) The patients having a poikilocytosis of 1 0 per cent and over had a significantly higher mean serum creatinine level than had those whose poikilocytosis was below 1 0 per cent

The incidence of MHA poikilocytosis was significantly affected by neither the duration of analgesic abuse nor by its discontinuance

There was an association between MHA poikilocytosis and reticulocytosis (r = + 0 33) and the former increased significantly with the rate of bone marrow erythropoiesis

MHA poikilocytosis was related neither to abnormal osmotic resistance of erythrocytes, nor to erythrocyte life span, serum iron concentration, haptoglobin concentration, or serum bilirubin As compared with patients with MHA poikilocytosis below 1 0 per cent, those having MHA poikilocytosis of 1 0 per cent and over showed a significantly higher mean reticulocyte value, a significantly greater frequency of increased bone marrow erythropoiesis, a significantly higher mean serum iron level, as well as significantly higher mean serum bilirubin There was also a shortening of red cell survival time and lowering of the haptoglobin concentration in patients with MHA poikilocytosis of 1 0 per cent or higher, but the differences were not significant

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Turku, February 1968

Jorma Forsstrom

APPENDIX

Analysis of variance with one or two-way classification was mainly used in the statistical treatment and the χ^2 test for testing of contingency hypothesis

Let the material be classified according to two classification principles A, (A_1, A_2, A_r) and B, (B_1 B_2, B_s), the two dimensional frequency distribution being presented as a contingency table Let v_{ik} denote the number of cases falling within the cell combination A_iB_k ($i = 1, 2,$ r and $k = 1, 2,$ s) Let

$$v_i = \sum_{k=1}^{s} v_{ik}, \; v k = \sum_{i=1}^{r} v_{ik} \text{ and } n = \sum_{i=1}^{r} \sum_{k=1}^{s} v_{ik}$$

The hypothesis H_0, to be tested, states that the classification principles A and B are stochastically independent, i e $P(A_iB_k) = P(A_i) P(B_k)$ It can be shown that the test variable

$$\chi^2_{(r-1)(s-1)} = \sum_{i=1}^{r} \sum_{k=1}^{s} \frac{\left(v_{ik} - \frac{v_i \; v_k}{n}\right)^2}{\frac{v_i \; v_k}{n}}$$

is χ^2 distributed with $(r-1)$ $(s-1)$ degrees of freedom if H_0 is correct If $\chi^2 \geqq \chi^2_p$, the null hypothesis is rejected at risk level p

In analysis of variance with two way classification we assume that the stratified sample is classified according to treatments A, (A_1, A_2, A_r) and B, (B_1, B_2, B_s) Let A_iB_k be the strata where A_i and B_k are applied The measured x value is then expressed as a linear model

$$x_{ijk} = \mu + \mu_i + \mu_j + \mu_{ij} + \varepsilon_{ijk}$$

in which it is known that

μ = general mean

μ_i = effect of method A_i

μ_j = effect of method B_j

μ_{ij} interaction and

random variations ε_{ijk} are (o, σ) normally distributed and independent

The residual variance σ^2 was estimated as

$$s_0^2 = \frac{\sum_{i=1}^{r} \sum_{j=1}^{s} \sum_{k=1}^{n_{ij}} (x_{ijk} - \bar{x}_{ij})^2}{\sum_{i=1}^{r} \sum_{j=1}^{s} n_{ij} - rs}$$

The hypothesis to be tested and the corresponding variance estimates are shown in the table below

Cause of variation	Hypothesis to be tested	Square sum	Degrees of freedom	Variance estimates
Methods A_i	H_1 $\mu_i = 0$	$\sum_{i=1}^{r} n_i (\bar{x}_i - \bar{x})^2 = Q_1$	$f_1 = r-1$	$s_1^2 = \frac{Q_1}{f_1}$
Methods B_j	H_2 $\mu_j = 0$	$\sum_{j=1}^{s} n_j(\bar{x}_j - \bar{x})^2 = Q_2$	$f_2 = s-1$	$s_2^2 = \frac{Q_2}{f_2}$
Interaction	H_3 $\mu_{ij} = 0$	$Q_3 = \sum_{i=1}^{r} \sum_{j=1}^{s} n_{ij}(\bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x})$	$f_3 = (r-1)(s-1)$	$s_3^2 = \frac{Q_3}{f_3}$

The test variable for the hypothesis H_l $(l=1, 2, 3,)$ is then $\frac{s_l^2}{s_0^2}$ and F-distributed with f_l and f_0 degrees of freedom if H_l is correct $(f_0 = \sum_{i=1}^{r} \sum_{j=1}^{s} n_{ij} - rs)$ If $F \geq F_p$, the effect to be tested is significant at risk level p

The practical calculations were carried out by computer in the Department of Applied Mathematics University of Turku

REFERENCES

ABBRECHT P H & GREENE J A JR Serum erythropoietin after renal homotransplantation Ann intern Med 65 908 1966

ABRAHAMS C, RUBENSTEIN A H LEVIN N W & WUNDERLICH U Experimentally induced analgesic nephritis in rats Arch Path *78* 222 1964

ADELSON L HEITZMAN E J & FENNESSEY J F Thrombohemolytic thrombocytopenic purpura Arch intern Med. *94* 42 1954

AHERNE W A The burr red cell and azotaemia J clin Path *10* 252 1957

ALLISON A. C. Acute haemolytic anaemia with distortion and fragmentation of erythrocytes in children Brit J Haemat *3* 1 1957

ANDEREGGEN P Ergebnisse der Sternalpunktion bei nephrogenen Anämien Helv med Acta *13* 3 1946

ANGERVALL, L., LEHMANN L. & BENGTSSON U The renal concentrating capacity in albino rats after long term consumption of phenacetin NAPA (N acetyl p-aminophenol) and acetyl salicylic acid Acta med scand *175* 155 1964

ANGERVALL, L. LEHMANN L. & LINCOLN K Induction of interstitial nephritis in rats fed phenacetin and NAPA (N acetyl p-aminophenol) Acta path microbiol scand *54* 274 1962

BECHER E Vorkommen und Ursachen der Anämie bei Nierenkrankheiten Münch med. *Wschr 77 1657 1930*

BELL, R E The origin of burr erythrocytes. *Brit J Haemat 9 552 1963*

BENGTSSON U A comparative study of chronic non-obstructive pyelonephritis and renal papillary necrosis Acta med scand Suppl *388* 1962

BENGTSSON U & HOOD, B Abuse of phenacetin containing analgesics and renal damage *In* Progress in pyelonephritis L. H Kass (ed) F A Davis Company Philadelphia 1965

BERMAN L. & POWSNER E R *Review of methods* for studying maturation of human erythroblasts in vitro Evaluation of a new method of culture of cell suspensions in a clot free medium Blood *14* 1194 1959

BOCK H. E BOTTNER H & SCHLEGEL, B Die Lebensdauer übertragener Erythrocyten bei Nierenkranken Ein Beitrag zur Pathogenese der nephrogenen Anämie. Z ges exp Med. *118* 459 1952

BOCK H E. LOHR G W & WALLER H D Beitrag zur Pathogenese renaler Anämien Schweiz med Wschr *92* 1213 1962 a

BOCK H E. NIETH H & SOLTH K Anämie bei Niereninsuffizienz Dtsch med Wschr *87* 573 1962 b

BOCK H E & THEDERING F Über Anämien bei Nierenkrankheiten II Mitteilung Dtsch Arch klin. Med *199* 130 1952

BOCK H E. WALLER H D KAUFMANN W & LOHR, G W Experimenteller Beitrag uber die Entstehung renaler Anämien Dtsch Arch klin Med *211* 73 1965

BOCK H F & WEYAND L Über Anämie bei Nierenkrankheiten I Mitteilung Die Blutmauserung bei Kranken mit blassem Hochdruck und solchen mit Übergangsformen von rotem zum blassem Hochdruck Dtsch Arch klin Med. *184* 369 1939

BONSDORFF E & JALAVISTO H A humoral mechanism in anoxic erythrocytosis Acta physiol scand *16* 150 1949

BRAIN M C Microangiopathic haemolytic anaemia. Thesis Oxford University 1963

BRAIN M C., DACIE, J V & HOURIHANE, D O Microangiopathic haemolytic anaemia The possible role of vascular lesions in pathogenesis Brit J Haemat *8* 358 1962

BRAIN M C., ESTERLY, J R & BECK E A. Intravascular haemolysis with experimentally produced vascular thrombi Brit J Haemat *13* 868 1967

BRAIN M C. & HOURIHANE D O Microangiopathic haemolytic anaemia The occurrence of haemolysis in experimentally produced vascular disease Brit J Haemat *13* 135 1967

BRECHER G & SCHNEIDERMAN M A time saving device for the counting of reticulocytes Amer J clin Path *20* 1079 1950

BRIGHT R. Reports of medical cases selected with view of illustrating the symptoms and cure of diseases by a reference to morbid anatomy London 1827 cit Verel *et al* 1959

BRODEUR M T H, SUTHERLAND D W KOLER R. D STARR A. KIMSEY J A. & GRISWOLD H L Red blood cell survival in patients with aortic valvular disease and ball valve prosthesis Circulation *32* 570 1965

BROWN G E & ROTH G M The anemia of

chronic nephritis Arch intern Med 30 817, 1922

BROWN G E & ROTH G M Prognostic value of anemia in chronic glomerular nephritis J Amer med Ass 81 1948 1923

BROWN R Plasma erythropoietin in chronic uraemia Brit. med J II 1036 1965

BROWN R Erythropoiesis in chronic renal disease Lancet II 319 1966

BUCHANAN J G Phenacetin induced chronic interstitial nephritis. N Z med J 60 207 1961

CALLEN I R & LIMARZI L R Blood and bone marrow studies in renal disease Amer J clin Path 20 3 1950

CAPELLI J P WESSON L G & ERSLEV A J Malignant hypertension and red cell fragmentation syndrome Ann intern Med 64 128 1966

CHAPLIN H & MOLLISON P L Red cell life span in nephritis and in hepatic cirrhosis Clin Sci 12 351 1953

CLAUSEN E Histological changes in rabbit kidneys induced by phenacetin and acetylsalicylic acid Lancet II 123 1964

CLAUSEN E Renal damage following long term administration of phenacetin and acetylsalicylic acid. Munksgaard, Copenhagen 1967

CLAUSEN E & HARVALD B Nephrotoxicity of different analgesics. Acta med. scand. 170 469 1961

CLAUSEN E & JENSEN K Renal biopsies on patients with a large abuse of analgesics A histological and bacteriological investigation In Abstracts of international congress of nephrology in Washington 1966

CLAUSEN E & PEDERSEN J Necrosis of the renal papillae in rheumatoid arthritis Acta med scand 170 631 1961

CONTRERA J F GORDON A S & WEINTRAUB A H Extraction of an erythropoietin producing factor from a particulate fraction of rat kidney Blood 28 330 1966

COOMBS R R A MOURANT A E & RACE R R A new test for the detection of weak and incomplete Rh agglutinins Brit J exp Path 26 255 1945

CZERNOBILSKY H & ERSLEV A J Erythropoietic activity of kidney extracts Clin Res. 14 314 1966

DACIE J V The haemolytic anaemias I—IV J & A. Churchill Ltd London 1954—1967

DACIE J V & LEWIS S M Practical haematology J & A. Churchill Ltd London 1963

DACIE J V MOLLISON P L RICHARDSON N SELWYN J G & SHAPIRO L Atypical congenital haemolytic anaemia Quart J Med 22 79 1953

DAWBORN J K KINCAID SMITH P & MCLAREN J The effect of aspirin and phenacetin on ascending infection in the rat kidney Aust Ann Med 13 217 1964

DESFORGES J F & DAWSON J P The anemia of renal failure Arch intern Med 101 326 1958

v DITTRICH P Glucose 6-phosphat Dehydrogenase in den Erythrocyten bei nephrogenen Anamien Klin Wschr 40 1075 1962

v DITTRICH P & SARTORIUS H Erfahrungen mit der extracorporalen Hamodialyse Klin Wschr 38 543 1960

DUNN J WEINSTEIN I M MAXWELL M H & KLEEMAN C R Significance of circulating phenols in anemia of renal disease Proc Soc exp Biol (N Y) 99 86 1958

EFFERSOE P Signs and symptoms in chronic renal failure Acta med scand 160 405 1958

EISALO A & TALANTI S Observations on the effect of phenacetin and N acetyl p-aminophenol on rat kidneys Acta med scand 169 655 1961

EMERSON C P JR & BURROWS B A The mechanism of anemia and its influence on renal function in chronic uremia J clin Invest 28 779 1949

ERSLEV A J Erythropoietic function in uremic rabbits Arch intern Med 101 407 1958

ERSLEV A J & HUGHES J R The influence of environment on iron incorporation and mitotic division in a suspension of normal bone marrow Brit J Haemat 6 414 1960

ESBACH J W JR FUNK D ADAMSON J KUHN I SCRIBNER B H & FINCH C A Erythropoiesis in patients with renal failure undergoing chronic dialysis New Engl J Med 276 653 1967

FAARUP C & OHLSEN S Sternalmarkuntersuchungen bei nephrogenen Anamien Folia haemat (Lpz) 67 152 1943

FIFIELD M M Renal disease associated with prolonged use of acetophenetidin containing compounds New Engl J Med. 269 722 1963

FINCH C A GIBSON J G PEACOCK W C & FLUHARTY R G Iron metabolism utilization of intravenous radioactive iron Blood 4 905 1949

FISHER J W & LANGSTON J W The influence of hypoxemia and cobalt on erythropoietin production in the isolated perfused dog kidney Blood 29 114 1967

FISHER J W SCHOFIELD R & PORTEOUS D D Effects of renal hypoxia on erythropoietin production Brit J Haemat 11 382 1965

FORDHAM C C HUFFINES W D & WELT L G Phenacetin induced renal disease in rats Ann. intern. Med 62 738 1965

FORSSELL J Nephrogenous polycythaemia Acta med scand 161 169 1958

FREY J Über Einwirkung von Phenacetin auf einige Stoffwechselabläufe der Mausetiere In Phenacetinabusus und Nierenschadigung H Sarre, A. Moench u R Kluthe (hrsg) Georg Thieme Verlag Stuttgart 1958

FRIIS T FOGH J & NISSEN N I Erytrocytlevetiden hos fenacetinspisere med og uden nyreinsufficiens. U geskr Laeg 121 1759 1959

Friis T Fogh J & Nissen N I The mechanism of reduction of erythrocyte lifetime in phenacetin habitués with renal impairment Acta med scand *168* 127 1960

Friis T & Nissen, N I The effect of phenacetin without acetic-4-chlor anilide on the erythrocyte lifetime in phenacetin habitués Acta med scand *173* 333 1963

Furuhjelm U Landtman B Laukmala K Nevanlinna H R Sulamaa M & Tuuteri L Hemolytisk anemi efter tillslutning av ostium primum-defekt Nord Med *72* 1446, 1964

Gallagher N I McCarthy J M Hart K T & Lange R D Evaluation of plasma erythropoietic stimulating factors in anemic uremic patients. Blood *14* 662 1959

Gallagher N I McCarthy J M & Lange R D Erythropoietic stimulating factor(s) and the anemia of chronic renal disease Clin Res *6* 391 1958

Gallagher N I McCarthy J M & Lange R D Observations on erythropoietic stimulating factor (ESF) in the plasma of uremic and nonuremic anemic patients Ann intern Med. *52* 1201 1960

Gasser C Gautier E Steck A Siebenmann R E & Oeschlin R Hamolytisch urämische Syndrome: Bilaterale Nierenrindennekrosen bei akuten erworbenen hamolytischen Anamien Schweiz med Wschr *85* 905 1955

Giovannetti S Balestri P L & Cioni L Spontaneous in vitro autohaemolysis of blood from chronic uraemic patients Clin Sci *29* 407 1965

Gloor F Die doppelseitige chronische nicht obstruktive interstitielle Nephritis Ergeb allg Path. path Anat. *41* 63 1961

Gloor F Phenacetinabusus und Nierenschadigung Schweiz. med Wschr *92 61 1962*

Gloor F Some morphologic features of chronic interstitial nephritis (chronic pyelonephritis) in patients with analgesic abuse *In* Progress in pyelonephritis L H Kass (ed) F A Davis Company Philadelphia 1965

Goldwasser E Fried W & Jacobson L Studies on erythropoiesis VIII The effect of nephrectomy on response to hypoxic anoxia J Lab clin Med *52* 375 1958

Gormsen J & Gjørup P A Anemia in chronic diseases of the kidneys Dan med Bull *2* 195 195

Grimlund K Phenacetin and renal damage at a Swedish factory Acta med scand Suppl *405* 1963

de Gruchy G C Clinical haematology in medical practice Blackwell Oxford 1964

Gsell, O Primar chronische interstitielle Nephritis in Beziehung zum Phenacetinabusus Bull schweiz Akad med Wiss *14* 124 1958

Gsell, O v Rechenberg H K & Miescher P Die primär chronische interstitielle Nephritis Dtsch med Wschr *82* 1673 1718 1957

Hadnagy Gs Gyergyay F & Antal, Z Beitrag zur Anämie der Nierenkranken Mitosenhaufigkeit in Dunndarm und Zungenepithel nach Nephrektomie Klin Wschr *43* 241 1965

Harvald B Har fenacetinholdige medikamenter betydning for opståelsen af kronisk pyelonefrit? Ugeskr Laeg *119* 1592 1957

Harvald B & Clausen E Nephrotoxity of acetylsalicylic acid. Lancet II 767 1960

Harvald B Valdorf Hansen F & Nielsen A Effect on the kidney of drugs containing phenacetin Lancet I 303 1960

Henry R J Sobel, Ch & Chiamori N On the determination of serum iron and iron binding capacity Clin chim Acta *3* 523 1958

Hirashima K & Takaku F Experimental studies on erythropoietin II The relationship between juxtaglomerular cells and erythropoietin Blood *20* 1 1962

Horisberger B, Grandjean E & Lanz F Untersuchungen uber den Medikamentenmissbrauch in einem Grossbetrieb der schweizerischen Uhrenindustrie Schweiz med Wschr *88* 920 1958

Hultengren N Renal papillary necrosis Acta chir scand *115* 89, 1958

Hutchison H E Jackson J M & Cassidy P Phenacetin induced haemolytic anaemia Lancet II 1022 1962

Jacobs L A & Morris J G Renal papillary necrosis and abuse of phenacetin Med J Aust *2* 531 1962

Jacobson L O Goldwasser E Fried W & Plzak L Role of the kidney in erythropoiesis Nature *179* 633 1957 a

Jacobson L O Goldwasser E Fried W & Plzak L The role of the kidney in erythropoiesis. Trans. Ass Amer Phycns *70* 305 1957 b

Jacobson L O Goldwasser E Gurney C W Fried W & Plzak L Studies of erythropoietin The hormone regulating red cell production Ann N Y Acad Sci *77* 551 1959 a

Jacobson L O Marks E K Gaston E O & Goldwasser E Studies on erythropoiesis XI Reticulocyte response of transfusion induced polycythemic mice to anemic plasma from nephrectomized mice and to plasma from nephrectomized rats exposed to low oxygen. Blood *14* 635 1959 b

Jasinski B Weitere Erkenntnisse zur Pathogenese der toxisch hamolytischen Anamie nach Phenacetin (Saridon) Schweiz med Wscr *80* 113 1950

Jasinski B & Muller D Zur Pathogenese der toxisch hämolytischen Anamien nach chronischen Phenacetinabusus. Schweiz med Wschr *80* 681 1950

JOSKE R A McALISTER J M & PRANKERD T A J Isotope investigations of red cell production and destruction in chronic renal disease Clin Sci 15 511 1956

JUVA K & LAURENT B Determination of the haptoglobin hemoglobin complex in human serum with aid of gel filtration. Scand J clin. Lab Invest Suppl 76 1963

KASANEN A Serum indican and endogenous indican clearance in renal insufficiency Ann Med intern Fenn Suppl 26 1958

KASANEN A The prognosis of kidney damage and anemia in the analgesic abuser Ann Med intern Fenn 56 93 1967

KASANEN A. FORSSTROM J & SALMI H A On the commonness causes and detrimental effects of the use of analgesics by factory workers and hospital patients Acta med. scand 172 15 1962

KASANEN, A KALLIO V & MARKKANEN T On serum uric acid and endogenic uric acid clearance in renal failure Acta med scand 160 503 1958

KASANEN A & KALLIOMAKI J L Correlation of some kidney function tests with hemoglobin in chronic nephropathies Acta med scand 158 213 1957

KASANEN A, KULONEN M & LEVANTO A Personal communication 1967

KASANEN A NANTO V & LEHTONEN A Urinary acid mucopolysaccharides in renal patients with and without abuse of phenacetin-containing analgetics Ann Med intern Fenn 53 33 1964

KASANEN A & SALMI H A Significance of some renal function tests in the early diagnosis of chronic pyelonephritis Acta med scand 165 147 1959

KASANEN A & SALMI H A The use of phenacetin by and its detrimental effects on a series of hospital patients Ann Med. intern Fenn 50 193 1961

KASANEN A & VASAMA R Njurpapillnekros Nord Med 73 101 1965

KAYE M The anemia associated with renal disease J Lab clin Med 52 83 1958

KINCAID SMITH P Pathogenesis of the renal lesion associated with the abuse of analgesics Lancet I 859 1967

KURATOWSKA Z LEWARTOWSKI B & MICHALAK E. Studies on the production of erythropoietin by isolated perfused organs Blood 18 527 1961

KURTIDES E S RAMBACH W A. ALT H L & DELGRECO F Effect of hemodialysis on erythrokinetics in anemia of uremia J Lab clin Med 63 469 1964

LAKEY W H Interstitial nephritis due to chronic phenacetin poisoning Canad med Ass J 85 477 1961

LARSEN K & MOLLER C E A renal lesion caused by abuse of phenacetin Acta med scand 164 53 1959

LATHE G H & RUTHVEN C R J Factors affecting the rate of coupling of bilirubin and conjugated bilirubin in the van den Bergh reaction J clin Path 11 155 1958

LEHTINEN M Mikroangiopaattinen hemolyyttinen anemia metastasoineen mahasyövän yhteydessä Duodecim (Helsinki) 83 1289 1967

LEVANTO A Personal communication 1966

LEVANTO A & FORSSTROM J Mikroangiopaattinen hemolyyttinen anemia (MHA) Duodecim (Helsinki) 80 73 1964

LEVIN N W RUBENSTEIN A H ABRAHAMS C JORDAAN J C & POSEL M M Phenacetin nephritis interstitial nephritis and necrotizing papillitis associated with chronic ingestion of phenacetin S Afr med J 36 553 1962

LINDENEG O FISHER S PEDERSEN J & NISSEN N I Necrosis of the renal papillae and prolonged abuse of phenacetin Acta med scand 165 321 1959

LINDVALL N Renal papillary necrosis Acta radiol (Stockh) Suppl 192 1960

LINMAN J W & BETHELL, F H Factors controlling erythropoieses Charles C. Thomas Springfield 1960

LOCK S P & DORMANDY K M Red-cell fragmentation syndrome A condition of multiple aetiology? Lancet I 1020 1961

LOGE J PH LANGE R D & MOORE C V Characterization of the anemia of chronic renal insufficiency J clin Invest 29 830 1950

LOGE J PH LANGE R D & MOORE C V Characterization of the anemia associated with chronic renal insufficiency Amer J Med 24 4 1958

LONG R J & UTZ D C Analgesic nephropathy Med Clin N Amer 50 1085 1966

LORENZEN I & SCHWARTZ M Phenacetin induced haemolysis in patients with chronic nephritis and phenacetin abuse Acta med. scand. 168 461 1960

LOWINGER S Die Erythropoese bei azotamischen Anamien Folia haemat (Lpz) 59 222 1938

LYNCH R C BAKKEN C L CASEY T H & ALFREY C P JR Microangiopathic hemolytic anemia in carcinoma of the stomach Gastroenterology 52 88 1967

MACGIBBON B H LOUGHRIDGE L W HOURIHANE D O B & BOYD D W Autoimmune haemolytic anaemia with acute renal failure due to phenacetin and p-aminosalicylic acid Lancet I 7 1960

MANDEL, H G & DAVISON C Nonnarcotic analgesics and antipyretics In Drill's pharmacology in medicine J R DiPalma (ed) McGraw Hill Book Company New York 1965

MANN D L DONATI R M & GALLAGHER N I Erythropoietin assay and ferrokinetic measurements in anemic uremic patients J Amer med Ass 194 1321 1965

FRIIS T, FOGH J & NISSEN N I The mechanism of reduction of erythrocyte lifetime in phenacetin habitués with renal impairment Acta med scand *168* 127, 1960

FRIIS T & NISSEN N I The effect of phenacetin without acetic 4-chlor anilide on the erythrocyte lifetime in phenacetin habitués Acta med scand *173* 333 1963

FURUHJELM U, LANDTMAN B, LAUNIALA K, NEVANLINNA, H R, SULAMAA, M & TUUTERI L Hemolytisk anemi efter tillslutning av ostium primum-defekt Nord Med *72* 1416 1964

GALLAGHER N I, McCARTHY J M, HART K T & LANGE R D Evaluation of plasma erythropoietic-stimulating factors in anemic uremic patients Blood *14* 662 1959

GALLAGHER, N I, McCARTHY J M & LANGE R D Erythropoietic stimulating factor(s) and the anemia of chronic renal disease Clin Res *6* 391 1958

GALLAGHER N I, McCARTHY J M & LANGE R D Observations on erythropoietic stimulating factor (ESF) in the plasma of uremic and nonuremic anemic patients Ann intern Med *52* 1201 1960

GASSER C, GAUTIER E, STECK A, SIEBENMANN R E & OBERHOLZER R Hämolytisch urämische Syndrome Bilaterale Nierenrindennekrosen bei akuten erworbenen hämolytischen Anämien Schweiz med Wschr *85* 905 1955

GIOVANNETTI S, BALESTRI P L & CIONI L Spontaneous in vitro autohaemolysis of blood from chronic uraemic patients Clin Sci *29* 407 1965

GLOOR F Die doppelseitige chronische nicht obstruktive interstitielle Nephritis Ergeb allg Path path Anat *41* 63 1961

GLOOR F Phenacetinabusus und Nierenschädigung Schweiz med Wschr *92* 61 1962

GLOOR F Some morphologic features of chronic interstitial nephritis (chronic pyelonephritis) in patients with analgesic abuse *In* Progress in pyelonephritis E H Kass (ed) F A Davis Company Philadelphia 1965

GOLDWASSER E, FRIED W & JACOBSON L Studies on erythropoiesis VIII The effect of nephrectomy on response to hypoxic anoxia J Lab clin Med *52* 375 1958

GORMSEN J & GJØRUP P A Anemia in chronic diseases of the kidneys Dan med Bull *2* 195 19

GRIMLUND K Phenacetin and renal damage at a Swedish factory Acta med scand Suppl *405* 1963

DE GRUCHY G C Clinical haematology in medical practice Blackwell Oxford 1964

GSELL, O Primär chronische interstitielle Nephritis in Beziehung zum Phenacetinabusus Bull schweiz Akad med Wiss *14* 124 1958

GSELL O, RECHENBURG H K & MIESCHER P Die primär chronische interstitielle Nephritis Dtsch med Wschr *82* 1673 1718 1957

HADNAGY Cs, GYERGYAY F & ANTAL, Z Beitrag zur Anämie der Nierenkranken Mitosenhäufigkeit im Dünndarm und Zungenepithel nach Nephrektomie Klin Wschr *43* 241, 1965

HARVALD B Har fenacetinholdige medikamenter betydning for opståelsen af kronisk pyelonefrit? Ugeskr Laeg *119* 1592 1957

HARVALD B & CLAUSEN E Nephrotoxity of acetylsalicylic acid Lancet *II* 767, 1960

HARVALD B, VALDORF HANSEN F & NIELSEN A Effect on the kidney of drugs containing phenacetin Lancet *I* 303 1960

HENRY R J, SOBEL, CH & CHIAMORI N On the determination of serum iron and iron binding capacity Clin chim Acta *3* 523 1958

HIRASHIMA K & TAKAKU F Experimental studies on erythropoietin II The relationship between juxtaglomerular cells and erythropoietin Blood *20* 1 1962

HORBBERGER B, CRANDJEAN L & LANZ F Untersuchungen über den Medikamentenmissbrauch in einem Grossbetrieb der schweizerischen Uhrenindustrie Schweiz med Wschr *88* 920 1958

HULTENGREN N Renal papillary necrosis. Acta chir scand. *115* 89 1958

HUTCHISON H L, JACKSON J M & CASSIDY P Phenacetin induced haemolytic anaemia Lancet II 1022 1962

JACOBS L A & MORRIS J G Renal papillary necrosis and abuse of phenacetin Med. J Aust. *2* 531 1962

JACOBSON L O, GOLDWASSER E, FRIED W & PLZAK L Role of the kidney in erythropoiesis Nature *179* 633 1957 a

JACOBSON L O, GOLDWASSER E, FRIED W & PLZAK L The role of the kidney in the production of erythropoiesis Trans Ass Amer Physns *70* 305 1957 b

JACOBSON L O, GOLDWASSER E, GURNEY C W, FRIED W & PLZAK L Studies of erythropoietin The hormone regulating red cell production Ann N Y Acad Sci *77* 551 1959 a

JACOBSON L O, MARKS E K, GASTON E O & GOLDWASSER E Studies on erythropoiesis XI Reticulocyte response of transfusion induced polycythemic mice to anemic plasma from nephrectomized mice *and to plasma from* nephrectomized rats exposed to low oxygen Blood *14* 635 1959 b

JASINSKI B Weitere Erkenntnisse zur Pathogenese der toxisch hämolytischen Anämie *nach* Phenacetin (Saridon) Schweiz med Wschr *80* 113 1950

JASINSKI B & MULLER D *Zur Pathogenese der* toxisch hämolytischen Anämien nach chronischen Phenacetinabusus *Schweiz* med. Wschr *80* 681 1950

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By

CURT FURBERG

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FROM THE DEPARTMENT OF CLINICAL PHYSIOLOGY (HEAD PROFESSOR HÅK
LINDERHOLM M D) UNIVERSITY OF UMEÅ UMEÅ SWEDEN

Effects of Beta-adrenergic Blockade on ECG, Physical Working Capacity and Central Circulation with Special Reference to Autonomic Imbalance

By

CURT FURBERG

UMEÅ 1968

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UMEÅ UNIVERSITET AND AB SCANMEDA GÖTEBORG

Contents

The present publication is based mainly on studies reported in the following papers

I FURBERG, C Adrenergic beta-blockade and electrocardiographical ST-T changes[1] Acta med scand, *181* 21, 1967

II BERGMAN, F and FURBERG, C Adrenergic beta-receptor blockade and ECG changes of organic origin Scand J clin Lab Invest, *19* 274, 1967

III FURBERG, C Effects of repeated work tests and adrenergic beta-blockade on electrocardiographic ST and T changes Acta med scand 1968 In print

IV FURBERG, C. Adrenergic beta-receptor blockade and the relationships between physical working capacity, heart volume, and the total amount of hemoglobin[1] Scand J clin Lab Invest, *19* 164, 1967

V FURBERG, C Adrenergic beta-blockade and physical working capacity Acta med scand, *182* 119, 1967

VI FURBERG, C and SCHMALENSEE, G v Beta-adrenergic blockade and central circulation during exercise in sitting position in healthy subjects Acta physiol scand 1968 In print

In the text these publications will be quoted as (I) and (II), etc

[1] Preliminary reports of these papers were given at the meeting of the Swed Soc. for Clin Physiol Stockholm Dec 1965

Introduction

It is commonly known that more or less pronounced ST and T depressions in the ECG may occur in apparently healthy subjects. Changes of the ST-T interval in the ECG have been reported by most investigators as occurring with greater frequency in patients with various mental disorders than in apparently healthy subjects. These ECG changes in subjects without signs of an organic heart disease are generally termed functional, vegetative or sympathicotonic. They are usually considered to be due to autonomic imbalance, primarily in the form of an increased sympathetic tone.

Many investigators have pointed out the similarity between the so-called functional ST and T changes and those known to occur in cases of coronary insufficiency and myocarditis, as well as the difficulty of differentiating between them. Electrocardiographic misinterpretations are liable to arise because of such difficulties.

It is also well-known that changes in the autonomic balance may be reflected as circulatory changes. An increase in pulse rate can be induced by various psychical stimuli. Tachycardia is a common sign especially in those patients suffering from mental disorders. A hyperkinetic circulation and sympathicotonic ECG changes characterize patients with vasoregulatory asthenia (Holmgren et al. 1957). These and similar circulatory disturbances occurring in subjects without signs of organic heart disease have generally been related to changes in the autonomic balance. They are similar to those observed in connection with adrenergic stimulation. It is of value, e.g. from a therapeutic point of view, to distinguish them from changes of other origins. Few reliable and easily performed methods are available for determining the degree of a functional circulatory disturbance.

The present investigation is concerned with some of the aspects of the above-mentioned diagnostic problems. Its general purpose has been to examine the influence of changes in sympathetic tone on some of the information obtained from a standardized work test. The effects of an adrenergic blockade have been used in the diagnosis of these changes. As adrenergic stimulation of the heart is mediated via the adrenergic beta-receptors (Ahlquist 1948) it was convenient to use an adrenergic beta receptor blocking agent in these studies.

The main questions which the investigation attempted to answer were:

1) Is it possible by means of a beta-adrenergic blockade to differentiate electrocardiographic ST and T depressions of so-called functional

origin from those of organic origin known to occur in patients with coronary insufficiency and myocarditis?

2) To what extent does the sympathetic tone influence the physical working capacity determined at a standardized work test in subjects with autonomic imbalance and in what way does a beta-blockade affect the determinants of the physical working capacity at pulse rate 170 beats/min?

CHAPTER I

Material and Methods

Material

The material consisted of 95 subjects, 64 men and 31 women. Nineteen are included in two separate studies, two subjects are included in three and two in four studies.

Study I included

1) Eleven subjects, aged 21 to 46 years, without case history, clinical or roentgenological signs of an organic heart disease but with electrocardiographic ST and T depressions recorded in connection with a standardized work test. A large proportion of the subjects could be described as anxious and tense and six had been treated with anxiolytics. Three subjects were suffering from slight cardiac symptoms consistent with the diagnosis of neurocirculatory asthenia (Noyes & Kolb 1963). A review of earlier ECG recordings for the subjects showed non systematic variations of the ST and T changes especially at rest and/or in the standing position. In all cases one ECG without any apparent ST and T depressions had been recorded after ST and T changes had first been observed. The subjects constituted a consecutive group of subjects who were willing to undergo a second work test, chest X rays and determination of the total amount of hemoglobin.

2) Twelve patients with angina pectoris according to WHO's criteria (Technical report 231 1962) and ECG changes during exercise typical of coronary insufficiency. They were between the ages of 44 and 62 and seven were included in a previous study of patients with angina pectoris (Jacobsson et al 1966). All patients were examined prior to the present investigation by means of repeated work tests. Recordings from these tests showed a typical ECG response to exercise on all occasions. In addition one of them had electrocardiographic signs of an old myocardial infarction.

3) Ten patients aged 12 to 61 years, who had a case history, clinical and/or roentgenological signs of myocarditis or sequela after myocarditis and electrocardiographic changes of the ST-T interval consistent with the diagnosis. Five patients were examined during the course of an acute myocarditis and in 5 patients the diagnosis was sequela after myocarditis. The latter patients were examined 1/2—13 years after the acute stage of their disease. In all but two cases a relationship between an infection and the occurrence of ST and especially T wave depressions on the ECG was observed and there was a typical development of the ECG changes. The diagnosis was considered to be suspect in two patients with sequela after myocarditis. For 1 1/2 years all patients sent to the laboratory and known by the author to fulfil the above criteria were included in this group.

Study II included 6 apparently healthy dogs of mixed breeds.

Study III included thirteen selected co-operative patients with various types of ST and T depressions on the ECG who were willing to carry out daily work tests for 4—6 days. The material consisted of eight subjects without signs of an organic heart disease and five patients with coronary insufficiency, myocarditis or sequela after myocarditis (criteria as in study I).

Study IV included twenty nine patients who made up a consecutive material of patients with mental disorders[1] examined in the laboratory by means of work tests before and during a beta blockade, chest X rays and determination of the total amount of hemoglobin. Patients with

[1]These patients were examined from a clinico-physiological point of view in collaboration with Dr Curt Morsing in the Department of Psychiatry.

an abnormal roentgenological heart volume or total amount of hemoglobin were excluded, as well as those with other signs of organic heart disease or somatic diseases The group consisted of patients with the following diagnoses schizophrenia 3 patients, endogenous depression 3, organic cerebral syndrome 2 psychogenic psychosis 1 depressive syndrome 3 anxiety state 5, neurasthenia 3 and neurocirculatory asthenia 9

Study V included forty one subjects with signs of hyperkinetic normokinetic or hypokinetic circulation The division into these groups was based on the relationships between physical working capacity at pulse 170 and the circulatory dimensions in the subjects and a comparison with regression equations from a group of healthy subjects (Holmgren et al 1959 a) Further criteria for selection were the same as in study IV Nineteen patients from study IV were included in this study All subjects with signs of hyperkinetic circulation who came to the author's notice over a period of 1½ years and who were willing to undergo further examinations were included in this study It also included five healthy volunteers with signs of a normokinetic circulation and 12 athletes who had signs of a hypokinetic circulation

Study VI included eight healthy male volunteers who were examined by means of a right heart catheterization Their ages were between 20 and 24

Further information about the material is given in the separate papers

Methods

The *electrocardiograms* (ECGs) at rest and during an orthostatic test were recorded with standard leads I II III unipolar extremity leads, aVR aVL aVF and precordial leads $CR_{1, 2, 4, 5, 7}$ and $V_{1, 4, 5, 7}$ Leads $CH_{4, 5, 7}$ with the indifferent electrode on the forehead (Sjostrand 1951 b Holmgren & Strandell 1961) were used during exercise on a bicycle ergometer After exercise when the subject rested in a supine position leads I II, III and $CR_{1, 2, 4, 5, 7}$ were used The ECGs were recorded by a direct writing ink jet recorder (Mingograph 42 or 81, Elema Schonander AB Stockholm) The ECG recordings were made using a paper speed of 50 mm/sec and the sensitivity was 10 mm for 1 mV The time constant of these ECG apparatuses was 2.5 sec. The P Q level was used as isoelectric level of the ECG

The following ECG patterns recorded during a standardized work test were considered typical of those patients with organic heart disease

Coronary insufficiency Appearance or accentuation of depressions of the ST segment of 2 mm or more during exercise in at least one lead Horizontal or downward sloping ST depressions and flat diphasic or inverted T waves were considered typical of the pattern during exercise (Fig 4 in paper I) Similar criteria for ST depressions during exercise were previously used by Sandberg (1961) and Areskog & Hallén (1964)

Myocarditis and sequela after myocarditis First type ST depressions and especially inverted T waves in at least one of the left precordial leads at rest During exercise less pronounced T wave inversions when the work load was increased Four min after work reappearance of the ECG pattern recorded at rest before the exercise (Fig 8 in paper I) *Second type* Appearance or accentuation of straight and slowly ascending horizontal or downward sloping ST depressions of at least 1 mm in the precordial leads during exercise at a heart rate of about 140 beats/min (Fig 4 in paper III) These patterns were previously described by Levander Lindgren (1952 1965) and Bengtsson (1957)

During the orthostatic test the subject stood leaning his neck against a wall for 8 min after which ECG and heart rate were recorded Such a test was a part of the standardized work test

An electrically braked bicycle ergometer (Holmgren & Mattsson 1954) was used in the work tests to determine the physical working capacity in the sitting position according to Sjostrand (1947) and Wahlund (1948) Females usually started working at a load of 200 kpm/min and males at 300 kpm/min The work load was then increased stepwise every 6th min

by 200 kpm/min for females and 300 kpm/min for males. Heart rate was determined after 2, 4 and 6 min at each load. The patients with coronary insufficiency continued the work tests until they felt precordial discomfort or pain to such a degree that they wanted to stop the work test. They were asked to work until they felt the same amount of precordial pain in the second test as they had felt in the first. The other patients in this study continued cycling until they felt unable to carry on any longer.

The physical working capacity at pulse 170 beats/min (W_{170}) was calculated by interpolation or extrapolation assuming a linear relationship between the work loads and the pulse rate after 6 min at each load. W_{150} was obtained in a similar manner. The work load at maximal working intensity was taken to be the heaviest load at which the subject worked for 6 min plus an increment proportional to the completed part of time at the highest work load (Strandell 1963). This increment was 33⅓ and 50 kpm for women and men respectively per min of work at the highest load.

Heart volume (HV) was determined in the prone position by the modified Larsson-Kjellberg method (1948). The heart volume was calculated principally by means of Jonsell's formula (1939) according to a method used by Linderholm & Strandell (1958).

The total amount of hemoglobin (THb) was determined according to the alveolar carbon monoxide method (Sjöstrand 1948) with slight modifications of the original method.

Cardiac output was determined during right heart catheterization according to the direct Fick principle. *Oxygen uptake* was measured using the Douglas bag technique. Samples of arterial and mixed venous blood were drawn off simultaneously during the collection of expired air.

Oxygen content was calculated after a spectrophotometric determination of hemoglobin concentration and oxygen saturation. *Blood pressure* was recorded on an Elema-Mingograf ECG apparatus using the Elema strain gauge mechano-electrical transducer.

Propranolol (Inderal) was used as the adrenergic beta receptor blocking agent. Ten mg was given orally to subjects weighing between 40 and 59 kg, 15 mg to those between 60 and 75 kg and 20 mg to those exceeding 75 kg. One patient with a body weight of 34 kg was given 5 mg. The work tests were started 1 hour after the administration of the drug (Shanks 1966) which was given, with few exceptions, about 2—3 hours after a meal.

Statistical calculations were mainly carried out according to Snedecor (1959). The correlation coefficients were calculated according to the methods of Pearson or Spearman. When two Pearson's coefficients of correlation were to be compared they were submitted to Fisher's z transformation. Differences between regression lines were tested according to Hald (1952). The following probability (P) levels of significance were used: $P \leq 0.001$ highly significant, $0.001 \leq P \leq 0.01$ significant and $0.01 \leq P \leq 0.05$ probably significant.

In the *animal experiments* myocardial damage was induced by poisoning dogs with orally administered thallium (Lannek 1949). The damage was verified histologically at the end of the experiment. ECGs were recorded by means of three bipolar standard leads and three bipolar precordial leads according to Lannek. The effect of a *beta blockade* on the ECG was studied five min after intravenous injection of propranolol in doses of about 0.20 mg/kg. Four experiments also included adrenergic stimulation by intravenous isoproterenol in a dose of about 2 µg/kg given within one min.

Further information about the methods is given in the separate papers.

Procedure

The patients usually performed a preliminary work test before propranolol was given. Within a week of the first examination a second work test was performed after the oral administration of propranolol. The same bicycle ergometer was used for each subject during these tests, which were performed in the same manner.

To avoid possible extracardial influence on the work tests, especially on the ST-T interval of the ECG, the tests were usually performed at the same time of day to avoid diurnal variation and about three hours after a meal to avoid

postprandial influences The patients were asked not to smoke for one hour before the work test No patient in the studies used, or had used for the last months digitalis, neuroleptics, antidepressive agents or other drugs known to significantly influence ECG or heart rate during exercise A few of the patients from the psychiatric department needed barbiturates in small doses Their therapy was not changed between the tests

Each patient in study III performed a series of work tests including 4—6 similar examinations Two of them in fact carried out eight and ten respectively The work tests were performed on consecutive days with a break during the holidays

The general procedure for the heart catheterization is described in detail in paper VI

Discussion

In the subjects without signs of organic heart disease different types of ST and T depressions were recorded on the ECG at rest, in the standing position and/or in connection with work The main characteristics of these ECG changes were their marked variability from normality to pronounced ST and T depressions They differed in most cases in some way from the typical patterns known to occur in patients with coronary insufficiency myocarditis or sequela after myocarditis These ST and T depressions seemed to be predominantly of a reversible nature

In the patients with signs of coronary insufficiency myocarditis and sequela after myocarditis ST and T depressions of different patterns were recorded in connection with a standardized work test These ECG changes varied inconsiderably at repeated recordings in most patients with coronary insufficiency The same was true for most of the patients who had myocarditic ECG changes of the second type In the rest of these patients there were variations in the ST and T depressions but in none of them was the typical ECG response to exercise altered The ECG changes in patients with acute myocarditis developed in a typical and systematic manner (Fig 9 in paper I) The ST and T depressions in the patients with organic heart disease were thought be of a type on the whole reproducible and different from that in patients without signs of organic heart disease In five patients with acute myocarditis the ECG changes later on completely regressed

The electrocardiographic ST and T depressions were localized in leads I II III, CR_{4-7} and V_{4-5} - ECG changes in patients with organic heart disease are predominantly localized in these leads and it is mainly ECG changes in these leads that may give rise to difficulties in differential diagnosis

The differences between recordings and determinations during work tests before and during beta adrenergic blockade were interpreted as due to propranolol It is possible that other factors might have contributed to these differences to some extent e g physical or psychical adaptation to the examination procedure This is difficult to determine and may vary between the individuals It was shown in paper III that ST and T depressions in the ECG in subjects without organic heart disease decrease gradually during a period of repeated work tests

W_{150} was preferred to W_{170} for a minority of the patients with coronary insufficiency or psychiatric diseases to avoid long extrapolations which may give uncertain values It was preferable especially during beta blockade when the highest recorded heart rates were about 10—20 beats/min lower than before the blockade

No systematic difference in W_{170} was found between work tests before and some days after the test with propranolol in twenty two patients included in study IV The mean value of W_{170} for 12 men was 946 kpm/min at the first test and 951 kpm/min at the third while the corresponding values for 10 women were 607 and 630 kpm/min The mean differences and their standard deviations were — 5 ± 145 kpm/min and — 23 ± 62 kpm/min respectively

In order to determine whether propranolol in fact influences the circulatory dimensions some complementary studies were made The heart volume in the supine position was determined for eight subjects in study VI The mean heart volume was 758 ml before and 752 ml during beta blockade The mean difference and it standard deviation was 6 ± 37

ml This difference is not significant It has been reported however that propranolol in larger doses causes a slight increase in the heart volume (Gebhardt et al 1965 Chamberlain 1966) The effect of propranolol on the THb (single determination) was examined in eleven subjects The mean value of THb was 426 g before and 421 g during beta blockade The difference is not significant The corresponding mean value at a third determination without the drug was 428 g Recently Morsing (1968) has found that propranolol affects the THb values in patients suffering from affective psychoses

Propranolol (1 isopropylamino 3 (1-napthyl-oxi)propranol 2) was introduced by Black et al (1964) In forearm blood vessels it blocks the vasodilation produced by epinephrine (Brick et al 1966) In the heart it blocks the chronotropic response of isoproterenol epinephrine and stellate ganglion stimulation and the inotropic response of isoproterenol and epinephrine It lacks sympathomimetic effects (Shanks 1966) The dose of propranolol about 0 22 mg/kg body weight orally used in the present study is somewhat small in comparison with that used in other investigations It was determined by testing the drug s capacity to abolish ST and T depressions in the ECG in a test group of subjects without signs of organic heart disease During the beta blockade there were no longer any ST and T depressions in the ECG

This dose of the drug had marked but not optimal effects on the heart rate A further decrease in the heart rate might have occurred if larger doses had been used This means that the above mentioned doses of propranolol do not necessarily completely block the pulse increase due to an increased sympathetic tone. It has been shown however by Åblad et al. (1967) that 0,26 mg propranolol/kg body weight administered orally blocks up to 83 % of the pulse rate increase after isoproterenol given intravenously in a dose of 1,5 μg per min Such a dose of isoproterenol is known to produce marked circulatory changes (Weissler Leonard & Warren 1959 Dodge Lord & Sandler 1960)

The present material consisted of patients aged 11 to 62 years with the majority between 18 and 42 It is known that the sympathetic and parasympathetic reactivity decreases with increasing age (Nelson and Gellhorn 1957) This means that certain results in papers IV—VI are not necessarily valid for children and elderly people

CHAPTER II

Beta-adrenergic blockade and electrocardiographic ST and T depressions

Previous investigations

Attempts to differentiate between electrocardiographic ST and T depressions of various origins by drugs were first made by Nordenfelt (1941) In his investigation he used ergotamine, a drug with a weak alpha adrenergic blocking effect and a pronounced vasoconstricting effect He concluded that if this drug abolishes ST and T changes in the ECG then these changes are "probably only functionally determined" ST and T depressions in patients with different organic heart diseases were, however, usually resistant to ergotamine Sometimes ergotamine caused a partial disappearance of the ST and T changes in these patients, which Nordenfelt explained by saying that sympathicotonic ECG changes may appear together with changes of a pathological nature

The observation that so-called "functional", sympathicotonic or reversible ST and T depressions in the ECG at rest more or less completely disappear after the administration of ergotamine, was later confirmed by several authors (Wendkos 1944, Spuhler 1947, Stroder 1950 Zeh 1950 and others) and on this point they seem to agree ST and T changes due to functional factors recorded during a hypoxaemia test (Biorck 1947) and during a step test (Master et al 1950) were also abolished after ergotamine Hydergine, the dihydrogenated analog of three ergot alkaloids in ergotoxine with a marked alpha-adrenergic blocking and a weak vasoconstricting effect, has a similar effect although not as great as that of ergotamine, on functional ST and T changes (Zeh 1950, Heimdal & Nordenfelt 1953)

Opinions are divided about the effect of ergotamine on ST and T changes in patients with organic heart diseases and about the value of the drug for differentiating between ECG changes of functional and organic origin Nordenfelt's opinion that the ergotamine test has a differential diagnostic value is shared by Spuhler (1947), Zeh (1950), Master et al (1950) and Stroder (1950) Leitner (1944) was of a similar opinion despite the fact that he found that a pathological ECG pattern indicating myocardial damage disappeared in 11 out of 54 patients He stated that autonomic lability often occurred in his patient material

A negative attitude to the use of ergotamine for differentiating between ST and T depressions of various origins was adopted by Scherf & Schlachman (1948), Kuhns (1949) and Steinmann, Kaufmann & Carnat (1951) Steinmann et al concluded that a separation of organic and neuro-vegetative ECG changes is "fundamentally impossible" Master et

al (1950) considered that by means of newer autonomic drugs it might be possible to 'provide accurate, safe, clinical methods for distinguishing functional from organic heart diseases

More recently it was reported that a ganglionic blockade with chlorisondamine caused a reduction in sympathicotonic ST and T depressions in patients with vasoregulatory asthenia (Arvedson, Furberg & Linderholm 1962, 1967) The effect of the drug on ECG changes in patients with coronary insufficiency was usually insignificant

In 1965 Nordenfelt reported that orthostatic ECG changes in healthy young subjects were inhibited by propranolol In the same year Friesinger et al reported that propranolol abolished the electrocardiographic abnormalities in a case with vasoregulatory asthenia In 1966 Suzman reported that ST and T depressions induced or accentuated in the standing position or during hyperventilation could be inhibited during a beta blockade in , anxiety states' Furberg & Tengblad (1966) found that propranolol prevents the occurrence of ST and T depressions which arise during hyperventilation in patients with psychiatric diseases

The effect of a beta adrenergic blockade on ECG changes in patients with angina pectoris has been reported briefly in a number of double-blind trials concerning the therapeutic effect of beta-blocking agents in cases of angina pectoris Srivastava Dewer & Newell (1964) Keelan (1965), Gillam & Prichard (1965) and Strait & Bruce (1965) pointed out that beta-blocking therapy did not affect ECG changes in patients with angina pectoris Adolfsson & Areskog (1967) found that the ECG reaction during a work test after the intravenous injection of propranolol in patients with coronary insufficiency was on the average somewhat less pathological, especially the changes at rest after work Dornhorst & Robinson (1962), Hamer et al (1964) and Apthorp, Chamberlain & Hayward (1964) reported that exercise changes in the ECG are less frequent and less severe in these patients after propranolol

Thus there are few systematic studies of the effect of beta-adrenergic blocking agents on ECG changes of various types and origins at rest, in the standing position and during and after work Studies of the effect of propranolol on ST and T changes in cases of myocarditis or sequela after myocarditis do not appear to have been published before the present work

Present investigations

A ST and T depressions in subjects without signs of organic heart disease (I, III)

Different types of ST and T depressions in the ECG were recorded in these subjects at rest, in the standing position and/or in connection with work They were localized in the standard leads and the left precordial leads The ECG changes disappeared during beta-blockade In a number of selected subjects with various types of ST and T depressions a similar effect was induced by 4—6 repeated work tests The following ECG patterns were recorded in these subjects before the beta blockade

ECG at rest (Fig 2 in paper III) The

ECG changes at rest usually consisted of only slight ST depressions with positive T waves In other cases more pronounced ST depressions with T wave inversions were recorded Both these types could be observed in the same individual on separate occasions When the T waves were diphasic the first component was usually negative and the second positive

In a few cases which appear to be more uncommon the ECG pattern chiefly consisted of T wave inversions These were isolated and recorded in one lead only, the apex lead, or localized in the left precordial leads The latter pattern which was recorded in one patient was connected with elevations of the ST interval

ECG during the orthostatic test (Fig 2 and 3 in paper III) The various types of ST and T depressions which were recorded at rest also appeared during the orthostatic test Here the changes were usually accentuated In some cases the ST and T changes appeared during this test

ECG in connection with the work test (Fig 1 and 2 in paper I and Fig 5 in paper III) The ST and T depressions which were recorded at rest usually decreased when the work loads were increased In certain cases an accentuation of the ST and T changes similar to that in the orthostatic test was observed when the subjects were working with lower work loads In these cases the ECG changes decreased at the highest work load Four min after the cessation of work the ECG usually reassumed the appearance it had had before the work when the subject was at rest or standing It sometimes happened that slight ST and T changes which were recorded at rest had disappeared 10 min after the work

B ST and T depressions in patients with signs of organic heart disease (I, III)

In patients with coronary insufficiency, myocarditis or sequela after myocarditis ST and T changes of different patterns were recorded during the work tests The *typical* ECG reactions in connection with work for these conditions (see Methods) were usually not, or only inconsiderably, affected by the beta-blockade and by the repeated work tests in five selected patients (Fig 4 in paper III)

Coronary insufficiency

ECG at rest (Fig 4—6 in paper I and Fig 4 in paper III) In a number of patients more or less pronounced ST and T depressions were recorded at rest In other cases there were no ST and T changes During the beta-blockade the slighter ST and T changes usually decreased, whilst the more pronounced ECG changes apparently remained unaffected by the blockade in a number of patients with advanced coronary insufficiency High positive T waves were recorded for some patients after propranolol

ECG during the orthostatic test (cf Fig above) In a few cases ST and T changes appeared or were accentuated during the orthostatic test These additional changes were blocked more or less completely by propranolol The

patients whose ECG changes at rest and in the standing position diminished after propranolol usually had a pronounced increase in W_{150} during the beta-blockade

ECG in connection with the work test (cf Fig above) All patients with coronary insufficiency had a typical ECG reaction during exercise before the blockade During the blockade the typical ST and T depressions often occurred at lower heart rates while the work loads were the same or higher in certain patients The ST and T depressions at the highest work load which was attempted were in most cases in the present study not, or only inconsiderably, affected by the blockade Five and eight repeated work tests did not influence the typical ECG response to exercise in two patients either Slight reductions of the ST and T depressions after propranolol were recorded for the others In all cases precordial pain was the reason for the interruption of the work tests Four min after work an accentuation of the T wave inversions was recorded before the blockade in certain patients with coronary insufficiency This change in the T wave which did not occur in all patients, was less pronounced or abolished after propranolol Ten min after work the ECG had usually reassumed the appearance it had had when the patient was at rest before the work

Myocarditis and sequela after myocarditis

Two different types of ECG reaction during the work test were recorded in the present investigation in these patients

The first pattern (Fig 8 and 9 in paper I and Fig 4 in paper III) was observed in six patients with acute myocarditis and two with sequela after myocarditis

ECG at rest and during the orthostatic test In all cases relatively pronounced T wave inversions were recorded in the left precordial leads at rest These were not affected or only inconsiderably so, by propranolol The T wave inversions were only inconsiderably influenced by 10 repeated work tests in one of these patients In some cases the T wave inversions were accentuated in the standing position This additional change was blocked by propranolol

ECG in connection with the work test As the work load was increased the T wave inversions for these patients decreased Four min after the work the T waves reassumed the appearance they had had when the patient was at rest before the work test. The ECG reaction during exercise was the same during the beta-blockade as before it

The second pattern (Fig 7 in paper I and Fig 4 in paper III) was recorded in one patient with acute myocarditis and in four with sequela after myocarditis

ECG at rest and during the orthostatic test The ECG was normal in four patients at rest and during the orthostatic test In one case (L E J), marked ST and T changes were recorded but they disappeared after five repeated work tests and during the beta-blockade (Fig 4 in paper III)

ECG in connection with the work test During heavy work, when the heart rate reached about 140—150 beats/min, various degrees of ST and T depressions occurred The heart rate was about the same for each patient before and during the beta blockade These ST and T depressions disappeared after the work This type of ECG pattern was not affected by the beta-blockade, nor by five repeated work tests in two patients

In the course of further ECG investigations, not yet published, another type of ECG pattern was observed in one patient with acute myocarditis It was in the case of a 15-year old girl where the T wave changes in the ECG at rest developed in a typical manner During an orthostatic test some weeks after the ECG at rest had become normal, T wave inversions reappeared and were of the same type as those observed at rest during the acute phase of the disease These T wave changes were not affected by propranolol They disappeared after a few months

C ST and T depressions in animals with verified myocardial damage (II)

The histological changes of the myocardium induced by thallium were on the whole similar to those seen in humans with toxic myocarditis or recent subendocardial infarctions (Fig 5 and 6 in paper II) The ST and T depressions that occurred after the damage was induced were not affected by propranolol (Fig 3 and 4 in paper II) Two of the poisoned dogs were given an adrenergic beta-receptor stimulating agent isoproterenol and in these cases the ST and T depressions increased (Fig 4) Such an increase in the ECG changes did not occur if the beta-stimulation took place after the dogs had been given propranolol

Discussion

1 ECG changes of functional origin

The ST and T depressions recorded in subjects without signs of organic heart disease seemed to be predominantly of a "reversible' nature and they completely disappeared during a beta-blockade These ECG changes were interpreted as sympathicotonic or functional and they were thought to reflect an autonomic imbalance The composition of the group of subjects with these ECG changes gives further support to this assumption Most of the subjects may be characterized as anxious and tense and six had been treated with anxiolytics

Most authors point out that patients with mental disorders have a higher frequency of ST and T changes than healthy subjects (Logue, Hanson & Knight 1944, Winton & Wallace 1946, Heyer, Winans & Plessinger 1947, Ruskin, Ravel & Beard 1947, White, Cohen & Chapman 1947, Major 1948, Veflingstad 1948, Plice & Pfister 1950, Rørvik & Aarstrand 1950, Blom 1951, Levander-Lindgren 1962) Oltman & Friedman (1951), on other hand, found no such difference It has also been shown that ECG changes are abolished or diminish in a patient group when the illness symptoms disappear (Plice & Pfister 1950 Magendantz & Shortsleeve 1951, Stevenson, Duncan & Ripley 1951, Levander-Lindgren 1964)

It is known that different psychical stimuli can affect the ECG and cause ST and T depressions This was shown by Mainzer & Krause (1940) in patients with fear before an operation Stevenson, Duncan & Ripley (1951), Magendantz & Shortsleeve (1951) and Mitchell & Shapiro (1954) and others have observed the appearance of ST and T changes in subjects with anxiety and tension Ljung (1952) could induce transient ST and T changes by frightening subjects in various ways during ECG recordings It is known that these stimuli may affect the autonomic balance It is probable that certain subjects who come for an ECG examination are exposed to similar stimuli They may be frightened by the investigation procedure itself because of all the electrodes (Ljung 1952) and may feel anxiety or fear about the result of the ECG examination and the consequences which may ensue

A period of physical training has previously been used in order to differentiate between sympathicotonic ST and T depressions and those of an organic nature Holmgren et al (1959 b) showed that sympathicotonic ST and T depressions in patients with vasoregulatory asthenia diminished or disappeared after physical training Levander Lindgren (1964) reported similar results from a study of a group of patients with neurocirculatory asthenia This group consisted of cases with low $W_{1\,0}$ as well as with ordinary W_{170}

The present investigations have shown that functional ECG changes at rest can be of different patterns The most common type consisted of ST depressions and more or less pronounced T wave depressions When the T wave was diphasic its appearance was similar to the one induced after the injection of epinephrine (Sjostrand 1951 a)

Isolated T wave inversions are usually not interpreted as being functional Littmann (1948), however, reported that T wave inversions in healthy young men are most apparent in the apex lead It is known that adrenergic stimulation with isoproterenol can cause myocardial damage in rats and that this is most often localized in the apex region (Rona et al 1959) This can be taken as a sign that this part of the heart is sensitive to sympathetic stimulation Isolated T wave changes were also observed by the present author in patients recovering from myocarditis and they were not affected by propranolol

An unusual type of functional T wave inversion is the one connected with ST elevations This type was previously observed in apparently healthy subjects by Grant, Estes Jr & Doyle (1951), Goldman (1960) and Blackman & Kuskin (1964) A similar pattern has been reported as occurring in patients with myocarditis (Levander-Lindgren 1952)

The subjects with ST and especially T wave depressions during an orthostatic test often had similar ECG changes four min after the work and these changes are, according to Lepeschkin (1951), probably of the same origin He also stated that the T depressions 3—5 min after work were most common in persons without physical training subject to early fatigue and in vegetatively labile persons

The present results about the effect of propranolol on ST and T depressions

connected with changes in the autonomic balance, occurs according to Hauss (1954) with at least as high a frequency among patients with coronary diseases as amongst subjects with no heart disease. Thus there are certain findings that indicate that ST and T changes related to autonomic imbalance may occur in these patients. It has, moreover, been pointed out in many studies that so called functional or vegetative ST and T depressions may occur simultaneously with depressions of organic origin (Nordenfelt 1941, Ljung 1949, Stevenson, Duncan & Ripley 1951, Bengtsson, Birke & Wingstrand 1951, Holmgren et al. 1959 b and others).

There are also investigations which indicate that ECG changes induced by adrenergic stimulation might be more common among patients with organic heart diseases. By means of animal experiments Bellet (1966) was able to show that a morphologically changed myocardium is more sensitive to adrenergic stimulation than a healthy one. Small doses of epinephrine produce ST and T changes in higher frequencies in patients with angina pectoris than in normal subjects (Lepeschkin 1951). Enger (1961) observed an increased sensitivity to different types of sympathetic stimulation in a patient some time after the acute stage of a myocarditis.

The patient's age should be taken into account here since the reactivity of the autonomic system appears to decrease with age (Nelson & Gellhorn 1957). Older people, for example, seldom have orthostatic ECG changes (Strandell 1963).

Coronary insufficiency

Katz, Hamburger & Lev (1932) showed that the ECG changes at rest in patients with coronary insufficiency increased by varying amounts after adrenergic stimulation with intravenous epinephrine. Similar results were found by Scherf & Schnabel (1934) during a vagal blockade which causes a deviation in the autonomic balance in a sympathetic direction. It was also shown that the ST and T depressions increase for some patients if they feel anxiety (Burch & Ray 1948) or fear (Mainzer & Krause 1940). Further support for the assumption that ST and T changes in some patients with organic heart diseases are increased via an increased sympathetic tone, was given indirectly by the observations that ST and T depressions at rest decrease for some patients with coronary insufficiency after vagal stimulation (Lepeschkin 1951), after bilateral thoracic sympathectomy (Apthorp, Chamberlain & Hayward 1964), and after X-ray irradiation of the adrenal glands (Raab & Schonbrunner 1939).

There are patients with coronary insufficiency whose ECG changes only appear in connection with an increased sympathetic tone and not when the patient's physical and mental balance is good (Sjostrand 1967). These ECG changes may, according to the same author, differ in pattern from the typical autonomic changes. Adrenergic stimulation with epinephrine was previously used for diagnostic purposes in order to prove the existence of coronary insufficiency (Katz, Hamburger & Lev 1932). It was shown that the oxygen consumption of the myocardium at rest

decreases for some patients during a beta-blockade, whereas the coronary blood flow is only slightly different (cf Fig 5 in Wolfson et al 1966) Thus it is reasonable to assume that ST and T changes at rest for some patients with *coronary insufficiency may diminish or* disappear after a beta-adrenergic blockade with propranolol

The above-mentioned studies have been used as a basis for interpretation of the propranolol effect on the ST and T changes observed in some patients with organic heart disease It was *thought that if ST and T depressions* diminish during a beta-blockade in these patients these depressions are probably of a functional' nature This judgement was also supported by some observations in the present study It was found that a marked decrease in heart rate during exercise usually occurred after propran*olol in the patients whose ECG changes* at rest and in the standing position diminished during the beta-blockade It was thought that the marked effect of propanolol on the heart rate indicated an autonomic imbalance

It has been shown that propranolol induces a significant decrease in the left ventricular work *during exercise in* patients with coronary heart disease (Åstrom 1968) The same is true for healthy subjects (VI) It seems probable that the diminution of ST and T depressions in some patients during beta-blockade corresponds to a reduced cardiac work Raab et al (1962) pointed out that an increased sympathetic tone leads to an augmentation of the cardiac work The favourable therapeutic effect of propranolol on most patients with coronary artery disease as well as the effect of the drug on ST and T depressions in certain patients may be due to the presence of an increased sympathetic tone in these patients

It is arguable whether or not the ST and *T depressions* that disappear during beta-blockade are to be interpreted as functional and in general to be distinguished from those ST and T depressions that are unaffected by the drug It can be assumed that the former reflect deviations in the autonomic balance and it would be possible, from this point of *view, to consider them as functional* There are, from a therapeutic point of view, certain reasons for distinguishing patients with signs of an increased sympathetic tone from other patients with angina pectoris A favourable effect of a beta-blocking agent would seem to be expected primarily in patients with an *increased sympathetic tone* (Furberg & Jacobsson 1967) It would be reasonable to expect a better therapeutic effect from physical training in patients with an increased sympathetic tone, as compared with patients with a predominantly parasympathetic tone, since physical training is thought to shift the autonomic balance in a *parasympathetic* direction

The ST and T depressions which were recorded at the highest work load decreased somewhat in a few cases in the present study after propranolol It is possible that an increased sympathetic tone affects the ECG even during heavy work in some patients and that the beta-blockade reduces somewhat their ST and T changes In no patient with coronary insufficiency in the present study

was the ECG reaction at work so affected by propranolol as to make the diagnosis difficult

Areskog et al (1967) consider, contrary to other authors, that T wave inversions after work may be just as specific a sign of considerable coronary insufficiency as prominent ST and T depressions during work The present author does not consider the T wave inversions recorded after work in some patients as being as typical of coronary insufficiency as the ST and T depressions during exercise To judge from the blocking studies it appears that the ST and T depressions during work are for the most part of a nature other than the T wave inversions recorded after work This assumption is supported by the investigations of Raab (1948) and Nestel, Verghese & Lovell (1967) Raab pointed out that patients with angina pectoris have a higher urinary excretion of 'epinephrine-like substances after work than healthy subjects Nestel, Verghese & Lovell said they had found a significant correlation between 'post-exercise electrocardiographic changes and the excretion of catecholamines in subjects with coronary artery disease'

Myocarditis and sequela after myocarditis

Functional ST and T changes also seem to occur simultaneously with ECG changes of organic origin in patients with myocarditis or sequela after myocarditis This was best illustrated in a patient whose marked ST and T depressions recorded at rest and in the standing position disappeared while those recorded during exercise remained after five repeated work tests

The ECG patterns for patients with myocarditis, which were recorded during the standardized work tests, varied in appearance before the blockade One reason for this may be that functional ST and T changes occurring together with those of organic origin may alter the patterns The number of types of ECG patterns recorded during a standardized work test decreased if the examination was carried out after propranolol

Beta-adrenergic blockade for differentiating between electrocardiographic ST and T depressions of functional and organic origin

The method suggested by Nordenfelt (1941) of using ergotamine for differentiating between ECG changes of different origins is used very little nowadays There appear to be two main reasons for this firstly because of ergotamine's adverse effects, especially the risk of serious complications if the drug is given to patients with coronary diseases (Scherf & Schlachman 1948, Master et al 1950), and secondly because of reports that ergotamine affects pathological ECG changes in organic heart diseases (Scherf & Schlachman 1948, Kuhns 1949, Steinmann, Kaufmann & Carnat 1951) Scherf & Schlachman stated that pathological T wave inversions were abolished at rest in five out of nineteen patients with different organic cardiac diseases Their investigation was made at rest To judge from the results

of the present study one could expect that five out of nineteen patients with organic heart diseases would have ECG changes of functional origin. It is possible that the results would have been different if the investigation had been carried out during a work test. The investigations of Kuhns and Steinmann, Kaufmann & Carnat cannot be commented on here as their results have not been presented in full.

In the present study it was shown that it is possible by means a beta adrenergic blockade to distinguish two types of ST and T depressions at rest, in the standing position and in connection with work. The first type was recorded in subjects without signs of organic heart disease. These changes completely disappeared during a beta blockade and they were interpreted as being of a functional origin. They were probably related to an increased sympathetic tone. The second type of ST and T depressions was recorded in patients with coronary insufficiency, myocarditis or sequela after myocarditis. In all these patients the typical ECG patterns for these conditions recorded at a standardized work test persisted on the whole after the administration of propranolol. These ECG changes were interpreted as being of an organic origin. In certain patients some ST and T depressions diminished during the blockade but in none of them was the typical ECG pattern recorded during a work test affected in a significant way so that the ECG diagnosis was complicated. This diminution was thought to be due to the fact that an increased sympathetic tone may exist in some patients with organic heart diseases and that this autonomic imbalance induces functional ECG changes that occur simultaneously with those of organic origin. These results indicate that beta-adrenergic blockades have a diagnostic value for differentiating between ST and T depressions of a functional origin and those of organic origin in patients with coronary insufficiency, myocarditis and sequela after myocarditis.

It seems that this method is of the greatest value when one wishes to know the nature of recorded ST and T depressions in subjects without signs of an organic heart disease. A complete disappearence of the ECG changes after propranolol indicates that they are of a functional origin and thus the risk of misinterpretation can be reduced here. Further support for this interpretation is provided if these ECG changes have shown a great variability, if the subjects heart rates at an orthostatic test are high and/or signs of a hyperkinetic circulation (a low W_{170} in relation to the circulatory dimensions) disappear during the beta-blockade. If ST and T depressions in subjects without signs of an organic heart disease remain unaffected by propranolol it seems that they are not of a functional origin and further ECG checks and observation are recommended. This seems to be valid especially when the ECG changes are 'reproducible' and if the subjects do not have signs of an increased sympathetic tone. If ST and T depressions are inconsiderably affected by the beta-blockade in patients with signs of organic heart disease this indicates that the ECG changes are of an organic origin. This interpretation is supported by the

presence during a work test of typical ECG patterns which are "reproducible", occurrence of subjective complaints during the work tests and lack of signs of an increased sympathetic tone If ST and T depressions disappear or diminish during a beta blockade in patients with more or less obvious signs of organic heart disease this indicates that the patients' ECG changes, to a greater or lesser degree express, an autonomic imbalance Further support for this interpretation is provided if these ECG changes have shown a great variability, if present signs of a hyperkinetic circulation disappear during the beta blockade and/or the patients do not have any subjective complaints during the work tests In the case of a partial disappearance of the ST and T depressions after propranolol, especially if the remaining ECG changes do not represent a typical pattern, another work test after the administration of propranolol may be called for Here one may increase the dose of propranolol by five mg ECG changes of organic origin are not affected by this higher dose of propranolol in the present author's experience It was, moreover, shown in the animal experiments that ECG changes of organic origin were not influenced by propranolol in a dose 4—5 times that used in humans It ought to be pointed out that double doses eliminate the occurrence of precordial pains during exercise in certain patients with coronary insufficiency (Adolfsson & Areskog 1967) A certain cautiousness is recommended with these patients during work tests with higher doses of propranolol

This method should be used principally for assessing the nature of ST and T changes It should not be used for determining whether or not a patient has an organic heart disease, since a clinical diagnosis should not usually be established solely on the basis of an ECG It seems that this method has a diagnostic value as a complement to other methods used in the diagnosis of heart diseases

CHAPTER III

Beta-adrenergic blockade, physical working capacity and central circulation during exercise

Previous investigations

Physical working capacity

Arvedson, Furberg & Linderholm (1962, 1967) found that W_{170} increased markedly in patients with vasoregulatory asthenia (VA) after chlorisondamine, a ganglionic blocking agent, while the corresponding increase for control subjects was insignificant After the ganglionic blockade the VA cases were able to perform somewhat heavier work, whereas the control subjects reacted in the opposite way Furberg, Koch & Östlund (1966) reported that in healthy subjects W_{170} was not significantly affected by chlorisondamine during exercise in the sitting or supine position

The first report about the circulatory effects of beta adrenergic blocking agents during exercise is to be found in the study by Dornhorst & Robinson (1962) They stated that the effect of a beta blockade on the heart rate during exercise was less for physically well-trained subjects than for those who were nervous and unaccustomed to work

In a step-test study, Bollinger, Gander & Forster (1965) found that the heart rate during exercise decreased from 170 to 137 beats/min after propranolol in patients with a tachycardia at rest and a low W_{170} in relation to the total amount of hemoglobin A similar although somewhat less pronounced decrease in heart rate was recorded in a control group The patients who were thought to have vasoregulatory asthenia were also able to perform heavier work during the beta-blockade, which was not the case for control subjects and patients with hyperthyroidism

Epstein et al (1965) found that the heart rate for submaximal and maximal work levels decreased by about 20% in healthy young men during the beta-blockade They also reported that the exercise endurance decreased after propranolol

In 1967 Thoren reported that the heart rate for a number of healthy boys, 9 and 11 years of age, was 12—16 % lower during submaximal work after propranolol He also pointed out that the maximal heart rate decreased whilst the capacity to perform the exercise was unchanged during the beta-blockade

Frick et al (1968) studied the effect of an autonomic blockade on the heart rate during work in healthy control subjects before and after a period of physical training After this period when the physical working capacity was increased the pulse decrease during the blockade was less than before

Central circulation during exercise

By means of treadmill studies of healthy young men Epstein et al (1965) found that propranolol caused significant decreases in heart rate, cardiac output and mean arterial pressure, whilst the calculated arterio venous oxygen difference increased Similar results were also reported by Cumming & Carr (1966) and Åstrom (1968) in studies of healthy young subjects during work tests in the supine position

Pronethalol, a beta-adrenergic blocking agent, which also has a sympathomimetic effect, influences cardiac output during exercise in the upright position only slightly in healthy subjects (Bishop & Segel 1963, Chamberlain & Howard 1964)

Arvedson, Furberg & Linderholm (1962, 1967) found that chlorisondamine decreased the heart rate and the cardiac output during work whilst the arterio-venous oxygen difference increased and the stroke volume remained unchanged in the VA patients Only insignificant changes were noted, however, for the control subjects

Present investigations (IV, V, VI)

W_{150} (W_{170}) and its relationship to the circulatory dimensions (IV)

The effect of propranolol on physical working capacity at a given pulse-rate, 150 and 170 beats/min (W_{150} and W_{170}), and its relationship to the circulatory dimensions heart volume and the total amount of hemoglobin were examined in a group of patients with various mental disorders Before the blockade they often had a low W_{150} (W_{170}) and these values deviated more from those calculated from the circulatory dimensions than in a group of healthy subjects (Holmgren et al 1959 a) During the beta blockade the W_{150} (W_{170}) increased and this increase was greatest for patients with a low W_{150} (W_{170}) in relation to values calculated from the heart volume and the total amount of hemoglobin After propranolol there was a closer relationship between W_{150} (W_{170}) and the circulatory dimensions For the group as a whole the deviations around the regression lines W_{150} HV and W_{150}-THb decreased by about $^1/_3$—$^1/_2$ and the correlation coefficients increased from about 0 6 to about 0 9 The above-mentioned relationships were also studied separately for men and women, and during the beta blockade the regression lines were not significantly different for female patients as compared with male patients

Physical working capacity in subjects with signs of hyper-, normo- or hypokinetic circulation (V)

The effect of propranolol on physical working capacity was compared for subjects with signs of hyper-, normo- and hypokinetic circulation In subjects with signs of hyperkinetic circulation who were thought to have vasoregulatory asthenia (VA), the W_{170} increased on average by about 80% during the beta-blockade The increase in W_{170} was significantly greater than that for the control subjects with signs of normokinetic circulation, and for a group of

athletes with signs of hypokinetic circulation In the control subjects the mean increase in W_{170} during the beta-blockade was about 20% and in the athletes about 10%

The effect of propranolol on W_{170} varied from case to case in the different groups Among both the control subjects and the athletes there were a few cases where the W_{170} increased more than the others and to only a slightly lesser degree than that of the VA cases Those athletes for whom there was a marked increase in W_{170} after propranolol were compared with the athletes for whom the increase was slight This comparison showed that the heart rate for the former cases increased during the orthostatic test more than that for the latter cases Six of the athletes took part in the Swedish Military Championships in ski-shooting Three of these were cases where there was a marked increase in W_{170} after propranolol, and they missed 6, 4 and 3 shots respectively out of the fifteen rounds which each of them fired The other three who took part in the championships and whose W_{170} increased only slightly after propranolol missed 2, 0 and 0 shots respectively

In the same study there was a significant correlation between the increase in W_{170} during the beta blockade on the one hand, and the pulse increase, or the pulse level, at an orthostatic test on the other

The VA cases, moreover, were able to perform somewhat heavier work during the beta blockade, whilst most of the athletes were not able to perform the same amount of work as before The differences between these groups were probably significant

Central circulation during exercise in the sitting position (VI)

In a catheterization study the effect of propranolol on the central circulation during exercise in the sitting position was examined in eight healthy young men The results of this study showed that propranolol caused a significant reduction in the heart rate, cardiac output, the oxygen saturation in mixed venous blood and the mean pressure in the brachial artery and a significant increase in the arterio-venous oxygen difference and the mean pressure in the pulmonary artery The oxygen uptake and the stroke volume were unchanged The W_{170} increased on average by 15 % during the blockade and the increase was correlated to the subjects heart rate at rest during an examination before the catheterization There was a similar correlation between the increase in W_{170} after propranolol and the difference in W_{170} between the examination before and the examination during the catheterization There was a greater increase in W_{170} after propranolol in those subjects who had a lower W_{170} during the catheterization than before it

Discussion

Physical working capacity

It is well known that the pulse rate and the blood pressure increase after various psychical stimuli (Best & Taylor 1955) Mostly it is anxiety and fear which induce these changes (cf Hilton 1965) Stevenson Duncan & Wolff (1949) demonstrated how the pulse rate

varies with changes in emotional states Circulatory disturbances have been reported as occurring at rest in patients with various mental disorders (McFarland & Huddleson 1936, Jones 1948, Cohen & White 1951, Feer 1962 and others) Signs of an increased sympathetic tone and hyperkinetic circulation at rest were reported for patients with psychoses and in 'chronic anxiety states' (Harris 1965 and 1966) This change was abolished by means of propranolol

Studies of the pulse-rate during exercise and the physical working capacity in cases of mental disorder were usually carried out on patients with neurocirculatory asthenia (Jones & Mellersh 1946, Jones 1948, Cohen & White 1951, Holmgren et al 1959 a, Levander-Lindgren 1962, 1963 and others) These patients often have a low physical working capacity (cf Levander-Lindgren 1963) A hyperkinetic circulation with a low physical working capacity at pulse rate 170 in relation to heart volume and the total amount of hemoglobin characterizes patients with vasoregulatory asthenia (VA) (Holmgren et al 1957) These patients often have subjective complaints similar to those suffering from neurocirculatory asthenia Morsing (1964) found a low W_{170} in 47 out of 147 hospitalized patients with various mental disorders This finding was most common among severe cases of anxiety states but occurred also among patients with schizophrenia or brain lesions These results were confirmed at the laboratory in a full-scale study comprising a consecutive material of 215 patients (Morsing 1967) With regard to the composition of the patient material in study IV it was reasonable to expect that there would be many patients with low W_{170} and W_{170} values before the blockade and a greater deviation in the relationships between W_{170} (W_{170}) and the circulatory dimensions than in healthy subjects

During the beta-blockade there was an increase in the physical working capacity at a given pulse-rate and closer relationships between W_{170} (W_{170}) and the circulatory dimensions The increases in W_{170} (W_{170}) were not accompanied by significant changes in the patients ability to perform heavy work The results of the blocking studies indicate that sympathetic tone influences W_{170} (W_{170}) and that certain patients with mental disorders have signs of an increased sympathetic tone

The increase in W_{170} after propranolol (V) was greatest in the subjects with signs of hyperkinetic circulation, i e in those with a low W_{170} in relation to HV and THb The effect of propranolol on W_{170} in these subjects was percentally about the same as Arvedson, Furberg & Linderholm (1967) found after chlorisondamine in patients with vasoregulatory asthenia The effect of propranolol was less pronounced in subjects with signs of a normokinetic circulation and was on an average least among athletes with signs of a hypokinetic circulation These results are in accordance with those of Frick et al (1968) Judging from their results it appears that physical training and beta-adrenergic blockades affect the circulation in a similar way, as can be seen from their effect on the heart rate

during exercise It is probable that they do this via the autonomic system by changing the autonomic balance in a parasympathetic direction It was previously reported by Holmgren et al (1959 a) that physical training normalizes the hyperkinetic circulation in patients with vasoregulatory asthenia

The determination of W_{150} or W_{170} and its relationships to the circulatory dimensions has proved to be a useful method for estimating the degree of sympathetic tone in various groups of patients The method was shown to have a practical application for predicting the therapeutic effects of a beta adrenergic blocking agent in groups of patients with angina pectoris (Furberg & Jacobsson 1967) and with neurocirculatory asthenia (Furberg & Morsing 1968) A favourable therapeutic effect in such patients may be expected to occur more often in patients with a low W_{170} in relation to W_{170}-values calculated from the heart volume or the total amount of hemoglobin

It seems that it may also be possible to get some idea of the degree of sympathetic tone by determining the pulse level and the pulse increase at an orthostatic test It must, however be taken into account that conditions known to be associated with a hyperkinetic circulation e g hyperthyroidism and anaemia (cf Wade & Bishop 1962), have to be excluded before the above methods for estimating changes in the autonomic balance may be used

All subjects in study V increased their W_{170} after propranolol which can be taken as a sign that a certain degree of sympathetic tone was present in all subjects A minority of the subjects with an ordinary or a high W_{170} in relation to the circulatory dimensions increased their W_{170} considerably during the blockade It is reasonable to assume that an increased sympathetic tone was present in these subjects This assumption is supported by the fact that these subjects had a greater increase in heart rate during the orthostatic test than the other athletes whose W_{170} increased only slightly during the beta blockade

The division of subjects in study V into groups with signs of a hyper-, normo- and hypo-kinetic circulation was supported by the differences in the pulse levels at rest and during an orthostatic test The patients in the first group had pulse rates of a magnitude reported to occur in VA patients (Holmgren et al 1957, Arvedson, Furberg & Linderholm 1967) Some of them also had sympathicotonic ECG changes The corresponding pulse rates were on an average lower in the control subjects with signs of a normokinetic circulation and lowest among the athletes with signs of a hypokinetic circulation

Thoren (1967) reported a higher percental increase in W_{170} after propranolol in healthy young boys than was found by the present author for the control subjects (V) and the healthy students (VI) Differences in the dosage of propranolol may have influenced the results and it is also probable that the sympathetic tone is somewhat greater in young boys than in young and middle-aged men

The maximal physical working capacity increased somewhat in the patients with signs of hyperkinetic circulation

whilst the opposite was true of the athletes No change was noted in the control group nor among the healthy students in study VI Bollinger, Gander & Forster (1965) reported a significant increase in the exercise endurance after propranolol in patients whom they thought had VA, while no increase was noted among control subjects In healthy young boys the capacity to perform work remained unchanged during the beta-blockade (Thoren 1967) Most of the boys subjectively experienced the work as being easier during the blockade Epstein et al (1965) reported that the exercise endurance at a work load that caused total exhaustion after 3—6 min in a group of healthy young men decreased by 40 % after propranolol Higher doses of propranolol were used by Epstein et al than was the case in the present studies and this may have contributed to differences in results

Sympathicotonic ST and T changes in the ECG are sometimes associated with a hyperkinetic circulation Such electrocardiographic and circulatory changes are characteristic of patients with vasoregulatory asthenia (Holmgren et al 1957 and 1959 b) Adrenergic stimulation with various drugs may induce both sympathicotonic ST and T changes (cf Lepeschkin 1951) and a hyperkinetic circulation at rest (Goldenberg et al 1948, Weissler, Leonard & Warren 1959, Dodge, Lord & Sandler 1960 and others) In the present study there were cases with both these types of changes There were also cases with signs of hyperkinetic circulation but with no ECG changes, as well as cases with functional ST and T wave depressions and a normal physical working capacity In study III the functional ECG changes disappeared after a period of 4—6 work tests whilst the W_{170} increased only slightly It appears that autonomic imbalance may be reflected in both sympathicotonic ST and T changes and hyperkinetic circulation which occur simultaneously or in isolation

Central circulation during exercise

The results of the catheterization study showed that propranolol in oral doses of about 0 22 mg/kg body weight reduced the cardiac output whilst the oxygen uptake remained unchanged i e the circulation became more hypokinetic during the beta-blockade The reduction in cardiac output was mainly caused by a *decrease in the heart rate whilst the* stroke volume was only slightly changed after propranolol The reduction in cardiac output was compensated for by an increased peripheral oxygen extraction which was evident from the increased arterio-venous oxygen difference These results are principally the same as those obtained by Epstein et al (1965), Cumming & Carr (1966), and Åstrom (1968), even if there is a quantitative difference which can be explained by differences in the dosage of propranolol and/or in the working position

Arvedson, Furberg & Linderholm (1967) showed that chlorisondamine normalized the hyperkinetic circulation during exercise in the VA patients while the effect on the control subjects was insignificant W_{170} increased by 80 % in the VA cases and by 10 % in the controls after chlorisondamine This

increase in W_{170} was made possible by a better oxygen utilization in the working muscles which was evident from the higher arterio venous oxygen difference In preliminary investigations the present author found that propranolol had a normalizing effect on the hyperkinetic circulation of VA patients which was similar to that of chlorisondamine These studies were made during exercise in the supine position It is known that there are differences in cardiac output and arterio-venous oxygen differences between exercise in the supine position and exercise in the sitting position (Bevegard, Holmgren & Jonsson 1960) This means that the above-mentioned results cannot be used with certainty to explain the effect of propranolol on W_{170} in the sitting position The present study showed that the increase in W_{170} in the sitting position after propranolol in healthy subjects can largely be explained by the fact that the arterio-venous oxygen difference increased whilst the other main determinant of W_{170}, the stroke volume, increased only slightly The circulatory effects of propranolol in the present study were similar to those induced by chlorisondamine and propranolol in the VA patients during exercise in the supine position The increase in the arterio-venous oxygen difference was, however, more pronounced in the VA cases There is no reason to believe that the circulatory effects of propranolol in various groups of subjects are other than quantitative Thus it seems probable that propranolol also causes an increase in the arterio-venous oxygen difference in patients with signs of a hyperkinetic circulation (V) and patients with various psychiatric diseases (IV) and that this explains the increases in W_{170} caused by the drug

The effect of propranolol on sympathicotonic ST and T depressions of the ECG and on a hyperkinetic circulation indicates that an increased sympathetic tone is one patho-physiological mechanism in the VA syndrome This is also supported by results from blocking studies of the peripheral and central circulation at rest which were carried out by Bollinger, Wirz & Luthy (1966)

In subjects with hyperkinetic circulation (V) it seems that the reduction in cardiac output was completely compensated for with the result that the subjects were able to perform at least the same amount of work during the blockade as before it A number of these subjects were able to perform more work after propranolol A slight decrease in the exercise endurance was seen among certain athletes It seems as if the reduction in cardiac output during the beta-blockade was not completely compensated for in all athletes Athletes usually have a high peripheral oxygen extraction and a high arterio-venous oxygen difference during heavy work which means that there can be little increase in this If the reduction in cardiac output is made more pronounced than in the present study (VI), e g by using larger doses of propranolol, it is reasonable to assume that the capacity to perform heavy work will be reduced This may explain the results reported by Epstein et al (1965) which showed that the exercise endurance diminished by 40 % during a beta-blockade They also found that the oxygen uptake decreased

somewhat at maximal work loads Thus it seems that the dose of propranolol and the kinetics of the circulation are factors which are of significance when assessing the effects of a beta-blockade on the capacity to perform heavy work

General summary and conclusions

The influence of autonomic imbalance on ECG, physical working capacity and central circulation was studied in ninety-five subjects between the ages of 11 and 62 by means of a beta-adrenergic blocking agent, propranolol The material included subjects with various signs of autonomic imbalance as well as patients with signs of organic heart disease The examinations comprised standardized work tests in the sitting position on a bicycle ergometer, chest X-rays and determinations of the total amount of hemoglobin and in eight subjects a right heart catheterization

In the first part of the investigation the author examined the possibility of using a beta-adrenergic blocking agent to differentiate between electrocardiographic ST and T depressions of so-called functional origin and those of organic origin in patients with coronary insufficiency and myocarditis

Different types of ST and T depressions were observed in a group of subjects without signs of organic heart disease These ECG changes which were recorded at rest, in the standing position and/or in connection with work showed a great variability at repeated examinations They were apparently of a 'reversible nature and they completely disappeared during a beta-adrenergic blockade It was shown in a selected group of subjects with different types of such ST and T depressions that the effect of 4—6 repeated work tests on the ECG changes was similar to that of propranolol It was characteristic of the subjects with these 'reversible ST and T depressions that they had other signs of autonomic imbalance such as a high heart rate at rest and in the standing position A number of these subjects had signs of a hyperkinetic circulation during exercise (low physical working capacity at pulse 170, W_{170}, in relation to the circulatory dimensions, heart volume and the total amount of hemoglobin) and these signs disappeared during the beta-blockade

Different types of ST and T depressions were recorded in patients with coronary insufficiency, myocarditis and sequela after myocarditis The ST and T depressions typical of these conditions that were recorded during a standardized work test were on the whole "reproducible The beta-blockade did not affect, or only inconsiderably influenced, the ST and T depressions in most of these patients These results were in accordance with those from animal experiments where myocardial damage was induced in dogs, and where it was shown that ST and T depressions of obvious organic origin

were not affected by propranolol In some patients with coronary insufficiency or myocarditis the ST and T depressions diminished during the beta-blockade Such patients usually had other signs of an autonomic imbalance e g a low W_{150} (W_{170}) in relation to the circulatory dimensions and these signs also disappeared during the beta-blockade The effect of a series of repeated work tests on the ST and T depressions was similar to that of propranolol in a selected group of such patients

Thus these ECG studies have shown that propranolol distinguished two types of ST and T depressions recorded in connection with a work test One type that completely disappeared during the beta blockade was recorded in subjects without signs of organic heart disease These ST and T depressions were interpreted as being of a functional origin The other type was recorded in patients with coronary insufficiency, myocarditis and sequela after myocarditis The typical ECG patterns recorded during a work test were on the whole unaffected by propranolol in these patients These ECG changes were interpreted as being of an organic origin In some patients with signs of autonomic imbalance there seemed to be ST and T depressions of both organic and functional origin The results indicate that a beta-adrenergic blockade is of diagnostic value for differentiating between electrocardiographic ST and T depressions of functional origin and those of organic origin in patients with coronary insufficiency, myocarditis and sequela after myocarditis

In the second part of the investigation the influence of sympathetic tone on the physical working capacity was examined by means of a beta-adrenergic blockade and this part also included an analysis of the effect of a beta-blockade on the determinants of W_{170}

In a study of patients with various mental disorders it was shown that they *often had a low physical working* capacity at pulse 150 and 170 (W_{150} and W_{170}) and that the individual W_{150} (W_{170}) values deviated greatly around the regression lines, which show the relationship between the physical working capacity and the circulatory dimensions During the beta-blockade the W_{150} (W_{170}) increased and the deviations around the regression lines decreased and became similar to those of healthy subjects

In another study it was shown that the increase in W_{170} during a beta-blockade was, on the average, greatest in patients with signs of hyperkinetic circulation i e a low W_{170} in relation to HV and THb and, on average, was lowest among athletes with a high W_{170} in relation to the circulatory dimensions It was characteristic of the former subjects that they had a high pulse rate at rest and in the standing position Some of them also had sympathicotonic ECG changes Such signs of autonomic imbalance were seldom observed in the athletes The increase in W_{170} during the beta-blockade was correlated to the pulse level or pulse increase during an orthostatic test It was also found that a few subjects with ordinary or high W_{170} increased their W_{170} markedly after propranolol They also had a greater in-

crease in heart rate during the orthostatic test as compared with the other subjects in these groups. The capacity to perform heavy work increased somewhat among the subjects with signs of hyperkinetic circulation while it decreased among most of the athletes during the beta-blockade.

In the final study which included right heart catheterization, it was found that propranolol significantly decreased cardiac output during exercise in the sitting position in healthy subjects. This was primarily caused by a decrease in the heart rate whilst the stroke volume was only slightly changed. The circulation became more hypokinetic during the beta blockade as the oxygen uptake did not change. The reduction in cardiac output and in mean pressure in the brachial artery induced by propranolol implies that cardiac work decreases during a beta-blockade.

Thus the results from the studies of physical working capacity and central circulation indicate that sympathetic tone may influence W_{150} and W_{170} in patients with various mental disorders as well as in healthy subjects. A low W_{170} in relation to the circulatory dimensions, HV and THb, and a high pulse level or pulse increase during an orthostatic test seem to be signs of an increased sympathetic tone. Signs of autonomic imbalance also seem to occur in a few subjects with ordinary or high W_{170}. The effect of the beta-blockade on the ability to perform heavy work seems to be correlated to the circulatory kinetics. The increase in W_{170} in the sitting position during a beta-blockade appears to be largely due to an increased arterio-venous oxygen difference while the other main determinant of W_{170}, the stroke volume, is only slightly influenced by the blockade.

References

ABLAD B JOHNSSON G, NORRBY, A & SÖLVELL, L Potency and time effect relationship in man of propranolol and H 56/28 - Comparative studies after oral administration Acta pharmacol 25 Suppl 2 85 1967

ADOLFSSON L & ARESKOG, N H Effekter av propranolol pa patienter med koronarinsufficiens Svenska Lak-Tidn 64 Suppl III 83 1967

AHLQUIST, R. P A study of the adrenotropic receptors Amer J Physiol 153 586 1948

APTHORP G H, CHAMBERLAIN D A & HAYWARD G W The effects of sympathectomy on the electrocardiogram and effort tolerance in angina pectoris Brit. Heart J 26 218 1964

ARESKOG N H BJÖRK L BJÖRK V O., HALLÉN A & STRÖM G Physical work capacity ECG reaction to work test and coronary angiogram in coronary artery disease Acta med scand Suppl 472 9 1967

ARESKOG N H & HALLÉN A ECG at rest and ECG response to exercise In Hallen A Angina pectoris Acta chir scand Suppl 323 70 1964

ARVEDSON O FURBERG C & LINDERHOLM H Effekt av ganglieblockad pa Ekg och fysisk arbetsformåga hos patienter med vasoregulatorisk asthenı Report given at the meeting of the Swed. Soc. of Cardiology Stockholm, Dec 1962

ARVEDSON O FURBERG C & LINDERHOLM H The effect of a ganglionic blocking agent (chlorisondamine) on electrocardiogram, physical working capacity and hemodynamics in patients with vasoregulatory asthenia Acta med scand Suppl 472 36 1967

ASTRÖM H Haemodynamic effects of beta-adrenergic blockade Brit Heart J 30 44 1968

BELLET S Adrenergic effects of nicotine on coronary blood flow and blood lipids In Prevention of Ischemic Heart Disease Charles C. Thomas Sprinfield 1966 p 74

BENGTSSON E Electrocardiographic studies in patients with abnormalities in serial examinations with standard leads during acute infectious diseases II Acta med scand 159 411, 1957

BENGTSSON E., BIRKE, G & WINGSTRAND H Acute non - specific myocarditis in scarlet fever and acute haemolytic tonsillitis Cardiologica 18 360 1951

BEST C H & TAYLOR, N B The physiological basis of medical practice Williams & Wilkins company Baltimore 1955

BEVEGARD S HOLMGREN A & JONSSON B The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume Acta physiol scand 49 279 1960

BISHOP J M & SEGEL, N The circulatory effects of intravenous pronethalol in man at rest and during exercise in the supine and upright positions J Physiol 169 112 P, 1963

BIÖRCK G Ergotamine and apparent coronary insufficiency Brit Heart J 9 181 1947

BLACK J W., CROWTHER A. F., SHANKS R. G., SMITH, L H. & DORNHORST A. C. A new adrenergic beta receptor antagonist. Lancet i 1080 1964

BLACKMAN N S & KUSKIN L Inverted T waves in the precordial electrocardiogram of normal adolescents Amer Heart. J 67 304, 1964

BLOM, G E A review of electrocardiographic changes in emotional states J nerv ment Dis 113 283 1951

BOLLINGER, A GANDER M & FORSTER G Pulsfrequenz und Leistungsfahigkeit vor und nach Beta Receptoren Blockade durch Propranolol Schweiz med Wschr 95 1075 1965

BOLLINGER A WIRZ P & LÜTHY, E Unterarmdurchblutung vor und nach Beta Receptoren blockade beim hyperkinetischen Herzsyndrm Bibl cardiol (Basel) 49 Suppl II 27 1966

BRICK I GLOVER E W HUTCHISON K J & RODDIE I C Effects of propranolol on peripheral vessels in man Amer J Cardiol 18 329 1966

BURCH G & RAY, T Cardiovascular system as the effector organ in psychosomatic phenomena J A M A 136 1011 1948

CARLSTEN A Orthostatic electrocardiogram after myocarditis Scand J clin Lab Invest 15 Suppl 76 54 1963

CHAMBERLAIN D A The haemodynamic effects of beta adrenergic blockade in man Cardiologia 49 Suppl II 27 1966

CHAMBERLAIN, D A & HOWARD J The haemodynamic effects of beta sympathetic blockade Brit Heart J 26 213 1964

COHEN M E & WHITE, P D Life situations emotions and neurocirculatory asthenia (Anxiety neurosis Neurasthenia Effort syndrome) Psychosom Med 13 355 1951

CUMMING G R & CARR, W Hemodynamic response to exercise after propranolol in normal subjects Canad J Physiol Pharmacol 44 465 1966

DODGE H T LORD J D & SANDLER H Cardiovascular effects of isoproterenol in normal subjects and subjects with congestive heart failure Amer Heart J 60 94 1960

DORNHORST A C & ROBINSON B F Clinical pharmacology of a beta adrenergic blocking agent (Nethalide) Lancet ii 314 1962

ENGER E Extracardiac influences on the electrocardiogram in organic heart disease Observations on a patient with myocarditis Amer Heart J, 62 553 1961

EPSTEIN, S E ROBINSON, B F KAHLER, R L & BRAUNWALD, E Effects of beta adrenergic blockade on the cardiac response to maximal and submaximal exercise in man J clin Invest 44 1745 1965

FEER H Schizophrenie und Blutkreislauf Psychopharmacologia 3 395 1962

FRICK M H ELOVAINIO R O & SOMER T The mechanism of bradycardia evoked by physical training Cardiologia 1968 In print

FRIESINGER, G C LIKAR, I BIERN, R O & MASON R E Vasoregulatory asthenia A cause for false positive exercise electrocardiograms (P) Circulation 32 Suppl II 90 1965

FURBERG C & JACOBSSON K A Propranolol and angina pectoris An analysis of the different responses to the drug in patients with angina pectoris Acta med scand 181 729 1967

FURBERG C KOCH G & ÖSTLUND H Die Wirkung einer Ganglien blockierenden Substanz auf die orthostatische Reaktion und die korperliche Leistungsfahigkeit in liegender und sitzender Stellung beim Normalen Arztl Forsch 20 305 1966

FURBERG C & MORSING, C Prognostik vid beta adrenergiskt blockerade terapi vid neurocirkulatorisk astheni 1968 To be published

FURBERG C & TENGBLAD, C F Adrenergic beta receptor blockade and the effect of hyperventilation on the electrocardiogram Scand J clin Lab Invest 18 467 1966

GEBHARDT W REINDELL, H KÖNIG K & BÜCHNER, C Herz und Kreislaufdynamik nach medikamentoser Beta Rezeptoren-Blockade Verh dtsch Ges Kreisl Forsch 31 99 1965

GILLAM P M S & PRICHARD B N C Use of propranolol in angina pectoris Brit med J 2 337 1965

GOLDENBERG M PINES K L BALDWIN E F GREENE D G & ROH C E The hemodynamic response of man to norepine

phrine and epinephrine and its relation to the problem of hypertension Amer J Med 5 792 1948

GOLDHAMMER S & SCHERF D Elektrokardiographische Untersuchungen bei Kranken mit Angina pectoris. Z. klin Med 122 134 1932

GOLDMAN M. J Normal variants in the electrocardiogram leading to cardiac invalidism Amer Heart J 59 71 1960

GRANT, R. P ESTES JR. E H. & DOYLE, J T Spatial vector electrocardiography The clinical characteristics of S T and T vectors. Circulation 3 182 1951

HALD A. Statistical theory with engineering applications. John Wiley & Sons, New York 1952

HAMER, J GRANDJEAN T MELENDEZ, L & SOWTON G E Effect of propranolol (Inderal) in angina pectoris Preliminary report. Brit med J 2 720, 1964

HARRIS W S On the mechanisms causing hyperkinetic circulation in chronic anxiety states J Lab clin Med. 66 875 1965

HARRIS W S Excessive cardiac adrenergic activity Cause of a hyperkinetic circulatory state in psychotic patients (P) Circulation 33/34 Suppl III 123 1966

HAUSS, W H Angina pectoris Thieme, Stuttgart 1954

HEIMDAL A & NORDENFELT O The effect of Hydergine on the electrocardiogram Cardiologia 23 359 1953

HEYER, H. E., WINANS H. M. & PLESSINGER, V I Alterations in the form of the electrocardiogram in patients with mental disease. Amer J med Sci 214 23 1947

HILTON S M Emotion Chapter 16 in The Physiology of Human Survival Academic Press London and New York 1965 p 465

HOLMGREN A JONSSON B., LEVANDER, M LINDERHOLM H. MOSSFELDT F., SJÖSTRAND T & STRÖM, G Effect of physical training in vasoregulatory asthenia, in Da Costa s syndrome and in neurosis without heart symptoms Acta med scand 165 89 1959 a.

HOLMGREN A., JONSSON B., LEVANDER, M LINDERHOLM, H., SJÖSTRAND T & STRÖM G Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (Vasoregulatory asthenia) Acta med scand 158 413 1957

HOLMGREN A JONSSON B., LEVANDER, M LINDERHOLM H SJÖSTRAND T & STRÖM G ECG changes in vasoregulatory asthenia and the effect of physical training Acta med scand 165 259 1959 b

HOLMGREN A & MATTSSON K. H. A new ergometer with constant load at varying pedalling rate Scand J clin. Lab Invest. 6 137 1954

HOLMGREN A & STRANDELL, T On the use of chest — head leads for recording of electrocardiogram during exercise Acta med scand 169 57 1961

JACOBSSON K. A KOCH G., LEVAN DER LINDGREN M & MICHAELSSON G Double blind trial with prenylamine (Segontin) in coronary insufficiency Acta med scand 180 129 1966

JONES M. Physiological and psychological responses to stress in neurotic patients J ment. sci 94 392 1948

JONES M. & MELLERSH, V A comparison of the exercise response in anxiety states and normal controls Psychosom Med 8 180 1946

JONSELL, S Method for determination of heart size by teleroentgenography (heart volume index) Acta radiol (Stockh) 20 235 1939

KATZ, L. N., HAMBURGER, W W & LEV, M The diagnostic value of epinephrine in angina pectoris Amer Heart J 7 371, 1932

KEELAN P Double blind trial of propranolol (Inderal) in angina pectoris Brit. med. J 1 897 1965

KÜHNS K Über den Wert des Ergotamine Testes fur die Beurteilung abnormer T Zacken im Elektrokardiogramm Cardiologia 15 64 1949

LANNEK N A clinical and experimental study on the electrocardiogram in dogs Ivar Haeggstroms Boktryckeri Stockholm 1949

LANNEK, N Kompendium i allman medicin vid Veterinarhögskolans avdelning for medicin

LARSSON H. & KJELLBERG S R. Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol (Stockh) *29* 159 1948

LEITNER, S J Untersuchungen uber die Gynergenprobe als neue elektrokardiographische Funktionsprufung Schweiz med Wschr *74* 1349 1944

LEPESCHKIN E. Modern electrocardiography The Williams & Wilkins company Baltimore 1951

LEVANDER LINDGREN M. Electrocardiographic studies in scarlet fever Acta paediat. Suppl 91 1952.

LEVANDER LINDGREN M. Studies in neurocirculatory asthenia (Da Costa s syndrome) I Variations with regard to symptoms and some pathophysiological signs Acta med. scand. *172* 665 1962

LEVANDER LINDGREN M. Studies in neurocirculatory asthenia III On the etiology and pathogenesis of signs in the work test and orthostatic test. Acta med scand *173* 631 1963

LEVANDER LINDGREN M. Studies in neurocirculatory asthenia (Da Costa s syndrome) IV Course during common treatment and physical training and relations between symptoms and signs Acta med scand *175* 791 1964

LEVANDER LINDGREN M. Studies in myocarditis IV Late prognosis Cardiologia *47* 209 1965

LINDERHOLM, H & STRANDELL, T Heart volume in the prone and erect positions in certain heart cases Acta med. scand *162* 247 1958

LITTMANN D Abnormal electrocardiograms in the absence of demonstrable heart disease. Amer J Med. *5* 337 1948

LJUNG O Ekg — Koronarinsuffizienz bei vegetativer Labilität Cardiologia *14* 191 1949

LJUNG O Ekg förandringar vid skramsel for registreringen Nord Med *48* 1227 1952

LOGUE R. B HANSON J F & KNIGHT, W A Electrocardiographic studies in neuro circulatory asthenia Amer Heart J *28* 574 1944

MAGENDANTZ H. & SHORTSLEEVE J Electrocardiographic abnormalities in patients exhibiting anxiety Amer Heart J *42* 849 1951

MAINZER, F & KRAUSE M. The influence of fear on the electrocardiogram. Brit Heart J *2* 221 1940

MAJOR, S The electrocardiogram in catatonic schizophrenia N Y St J Med *48* 1489 1948

MASTER, A M PORDY L, KOLKER, J & BLUMENTHAL, M The two step exercise electrocardiogram in functional heart disturb ances and in organic heart disease The use of ergotamine tartrate Circulation *1* 692 1950

McFARLAND R. A & HUDDLESON J H Neurocirculatory reactions in the psychoneuroses studied by the Schneider method. Amer J Psychiat *93* 567 1936

MITCHELL, J H. & SHAPIRO, A. P The relationship of adrenaline and T wave changes in the anxiety state Amer Heart J *48* 323 1954

MORSING C. Cardiovascular adaptation to physical work in psychiatric patients Report given at the 6th European conference on psychosomatic research, Athens 1964

MORSING C Cirkulationsfysiologiska syn drom vid psykiska sjukdomar Report given at the meeting of the Swed Soc for Clin Physiol., Stockholm Nov., 1967

MORSING C. The effect of beta adrenergic blockade on the determination of the total amount of haemoglobin in affective psychoses Acta psychiat. 1968 In print.

NELSON R. & GELLHORN E The action of autonomic drugs on normal persons and neuropsychiatric patients The role of age Psychosom Med. *19* 486 1957

NESTEL, P J VERGHESE A & LOVELL, R. R. H. Catecholamine secretion and sym pathetic nervous responses to emotion in men

with and without angina pectoris Amer Heart J 73 227 1967

NORDENFELT O Über funktionelle Veränderungen der P und T Zacken im Electrokardiogramm Acta med scand Suppl 119 1941

NORDENFELT O Orthostatic ECG changes and the adrenergic beta receptor blocking agent propranolol (Inderal) Acta med scand 178 393 1965

NOYES A P & KOLB L C Modern clinical psychiatry Saunders Co Philadelphia 1963

OLTMAN J E & FRIEDMAN S The electrocardiogram in psychiatric patients J nerv ment Dis 113 127 1951

PLICE S G & PFISTER C W An electrocardiographic study of psychiatric patients before and after electroshock therapy Amer Heart J 40 252 1950

RAAB W Adreno-sympathogenic heart disease (Neurohormonal factors in pathogenesis and treatment) Ann intern Med 28 1010 1948

RAAB W van LITH P LEPESCHKIN E & HERRLICH H Catecholamine induced myocardial hypoxia in the presence of impaired coronary dilatability independent of external cardiac work Amer J Cardiol 9 455 1962

RAAB W & SCHÖNBRUNNER E Die Normalisierungstendenz des Elektrokardiogrammes nebennierenbestrahlter Angina pectoris Kranker Arch Kreisl Forsch 4 362, 1939

RISEMAN J E F WALLER J V & BROWN G M The electrocardiogram during attacks of angina pectoris its characteristics and diagnostic significance Amer Heart J 19 683 1940

RONA G CHAPPEL C I BALAZS T & GAUDRY R An infarct like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat Arch Path 67 443 1959

RØRVIK K & AARSTRAND T Elektrokardiografiske forandringer ved angstpreget nevrose Nord Med 43 950 1950

RUSKIN A RAVEL J & BEARD B The electrocardiogram in mental disease Tex Rep Biol Med 5 232 1947

SANDBERG L Studies on electrocardiographic changes during exercise tests Acta med scand Suppl 365 1961

SCHERF D & SCHLACHMAN M Electrocardiographic and clinical studies on the action of ergotamine tartrate and dihydro-ergotamine 45 Amer J med Sci 216 673 1948

SCHERF D & SCHNABEL P Atropin bei Angina pectoris Klin Wschr 13 1397 1934

SHANKS R G Pharmacological studies with propranolol (Inderal) Cardiologia 49 Suppl II 1 1966

SIMONSON E Differentiation between normal and abnormal in electrocardiography C. V Mosby Co St. Louis 1961

SJÖSTRAND T Changes in the respiratory organs of workmen at an ore smelting works Acta med scand Suppl. 196 687 1947

SJÖSTRAND T A method for the determination of the total haemoglobin content of the body Acta physiol scand 16 211 1948

SJÖSTRAND T After potentials in the electrocardiogram Acta physiol scand 24 247 1951 a

SJÖSTRAND T The electrocardiographic work and hypoxemia tests Scand J clin Lab Invest 3 1 1951 b

SJÖSTRAND T Electrocardiography Chap XXI in Clinical Physiology Bonniers, Stockholm 1967

SNEDECOR, G W Statistical methods Iowa State College Press Iowa 1959

SPÜHLER, O Die experimentelle Untersuchung eines neuen Sympathicolyticums des Dihydroergotamins (DHE 45) Schweiz med Wschr 77 28 1947

SRIVASTAVA S C DEWAR, H A & NEWELL, D J Double blind trial of propranolol (Inderal) in angina of effort Brit. med J 2 724 1964

STEINMANN B., KAUFMANN P & CARNAT, G Über die Frage der Unterscheidung einer Herzmuskelschädigung von neuro-

LANNEK, N A clinical and experimental study on the electrocardiogram in dogs Ivar Haeggstroms Boktryckeri Stockholm 1949

LANNEK, N Kompendium i allman medicin vid Veterinarhogskolans avdelning for medicin

LARSSON, H & KJELLBERG, S R Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol (Stockh) 29 159, 1948

LEITNER S J Untersuchungen uber die Gynergenprobe als neue elektrokardiographische Funktionsprufung Schweiz med Wschr 74 1349 1944

LEPESCHKIN E Modern electrocardiography The Williams & Wilkins company, Baltimore 1951

LEVANDER LINDGREN M Electrocardiographic studies in scarlet fever Acta paediat. Suppl 91 1952

LEVANDER LINDGREN M Studies in neurocirculatory asthenia (Da Costa s syndrome) I Variations with regard to symptoms and some pathophysiological signs Acta med scand 172 665 1962

LEVANDER LINDGREN M Studies in neurocirculatory asthenia III On the etiology and pathogenesis of signs in the work test and orthostatic test Acta med scand 173 631, 1963

LEVANDER LINDGREN M. Studies in neurocirculatory asthenia (Da Costa s syndrome) IV Course during common treatment and physical training and relations between symptoms and signs Acta med scand 175 791 1964

LEVANDER LINDGREN M Studies in myocarditis IV Late prognosis Cardiologia 47 209 1965

LINDERHOLM H & STRANDELL, T Heart volume in the prone and erect positions in certain heart cases Acta med scand 162 247 1958

LITTMANN D Abnormal electrocardiograms in the absence of demonstrable heart disease Amer J Med. 5 337 1948

LJUNG O Ekg — Koronarinsuffizienz bei vegetativer Labilitat Cardiologia 14 191 1949

LJUNG O Ekg forandringar vid skrämsel for registreringen Nord Med 48 1227, 1952

LOGUE R B HANSON J F & KNIGHT, W A Electrocardiographic studies in neurocirculatory asthenia Amer Heart J 28 574 1944

MAGENDANTZ H & SHORTSLEEVE J Electrocardiographic abnormalities in patients exhibiting anxiety Amer Heart J 42 849, 1951

MAINZER F & KRAUSE M The influence of fear on the electrocardiogram Brit Heart J 2 221 1940

MAJOR S The electrocardiogram in catatonic schizophrenia N Y St J Med 48 1489 1948

MASTER A M PORDY L KOLKER J & BLUMENTHAL M The two step exercise electrocardiogram in functional heart disturbances and in organic heart disease The use of ergotamine tartrate Circulation 1 692 1950

McFARLAND R A & HUDDLESON, J H. Neurocirculatory reactions in the psychoneuroses studied by the Schneider method Amer J Psychiat 93 567 1936

MITCHELL, J H & SHAPIRO A P The relationship of adrenaline and T wave changes in the anxiety state Amer Heart J 48 323 1954

MORSING C Cardiovascular adaptation to physical work in psychiatric patients Report given at the 6th European conference on psychosomatic research Athens 1964

MORSING C Cirkulationsfysiologiska syndrom vid psykiska sjukdomar Report given at the meeting of the Swed Soc for Clin Physiol Stockholm Nov 1967

MORSING C The effect of beta adrenergic blockade on the determination of the total amount of haemoglobin in affective psychoses Acta psychiat 1968 In print

NELSON R & GELLHORN E The action of autonomic drugs on normal persons and neuropsychiatric patients The role of age Psychosom Med 19 486 1957

NESTEL P J, VERGHESE A & LOVELL, R R H Catecholamine secretion and sympathetic nervous responses to emotion in men

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 489

FROM THE DEPARTMENT OF MEDICINE I (PROFESSOR L. WERKÖ) SAHLGRENSKA HOSPITAL, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

LUNG MECHANICS IN RHEUMATIC VALVULAR DISEASE

BY

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GÖTEBORG 1968

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GÖTEBORG 1968
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CONTENTS

AIM OF PRESENT STUDY

The object of the present study was to analyze in a series of patients with rheumatic valvular disease how the lung mechanics including lung volumes maximum expiratory flows, lung compliance and lung conductance, were related to the symptoms appearance of pulmonary roentgenograms roentgenographic heart volume pulmonary vascular pressures and resistance and pulmonary blood volume

The apparatus used for this study had not yet been checked other than by the manufacturers and this was therefore done before the subjects were examined. A control series was studied as normal values were lacking for some of the tests used and it was not known how some of them are affected by age and anthropometric variables The study was done according to the following plan

(a) *Arrangement of an apparatus for* measuring lung volumes compliance conductance (resistance) and maximum expiratory flows (flow volume curves) Testing the properties of the apparatus and evaluating their capacity to measure these variables

(b) Study of the effect of age and anthropometric values on the lung volumes, compliance, conductance and maximum expiratory flows in a group of *nonsystematically* chosen men and women Comparison of certain variables with available data from randomly selected inhabitants of the city of Goteborg and also comparison with data from other large series not chosen completely at random

(c) Comparison of the ventilatory function and lung mechanics *in mitral* and aortic valvular disease with the degree of disability the pressure and flow in the pulmonary circulation, the pulmonary blood volume, and the size of the heart consideration being given to age and anthropometric variables affecting the values for tests of the foregoing Study of the effect of diuretic therapy on the ventilatory function and pulmonary mechanics

REVIEW OF LITERATURE

Dyspnea is common in heart disease Other familiar symptoms in heart disease especially mitral stenosis, are coughing expectoration and wheezing Yet not until the present decade has a systematic study been made of these symptoms in heart disease Thus in 1963 Aber, studying the occurrence of recurrent bronchitis in a series of 84 patients with mitral stenosis found that the rate of cough expectoration and wheezing increased with the degree of functional disability

Morphologic changes in lungs in heart disease

In 1935 Brenner described the morbid anatomic changes occurring on increase in pulmonary venous pressure and the following year Parker & Weiss (1936) described these changes more in detail Among the more recent descriptions is that of Gough (1960) The prominent observations have been hemosiderosis presumed to be caused by intrapulmonary hemorrhage together with swelling of the alveolar cells and thickening of the basement membrane (Schulz 1962) tortuous and dilated lymphatic vessels in the lung (Heath & Hicken, 1960) thickening and edema of the interlobular septa presumed to be the cause of the Kerley B lines in pulmonary roentgenograms (Gough 1955) dilation of the main and lobar pulmonary arteries no dilation of the smaller elastic vessels or only a slight amount in the upper lobes—in the lower lobes where the media and intima of the small muscular arteries are thickened, these vessels are sometimes narrowed (Harris & Heath 1962)

Several have examined the relationship between the findings on lung biopsy and the pressure in the pulmonary circulation in mitral stenosis (Cloves, Hackel & Mueller 1953 Curti Cohen Castleman, Scannell Friedlich & Myers 1953 Jordan Hicken Watson Heath & Whitaker 1966) Jordan *et al* (1966) studying lingular biopsies reported. no relation between the degree of medial hypertrophy in the vessels and the degree of the pulmonary vascular resistance a relation between horizontal lines in pulmonary roentgenograms and the occurrence of alveolar fibrosis but not with dilated lymphatic vessels no relation between changes in the alveolar walls and the respiratory function.

Effect of age and anthropometric variables on the lung mechanics

Studies of the ventilatory function and pulmonary mechanics in different diseases must take into account all the factors that normally affect these conditions Among the early studies of such factors are those of Hutchinson (1846) and Arnold (1855) who examined the relationship between vital capacity and height among other things In 1961 Mead published a summary of most of what was known up to then on the compliance and resistance of the lungs and on the factors such as lung volumes affecting these variables

During recent years further facts on the mechanical properties of the lungs have emerged from studies of the maximum expiratory flows in flow volume curves (Hyatt, Schilder & Fry, 1958) Mead Turner, Macklem & Little (1967) studied the effect of age on these curves

Many have studied the effect of smoking on pulmonary function, but it has been impossible to determine the effect of air pollution outside the laboratory on the lungs

The normal ranges of the various lung volumes variables of ventilatory function and pulmonary mechanics and how certain factors affect these variables are described in Handbook of Physiology; Respiration (Fenn & Rahn 1964–1965)

Lung mechanics in heart disease

Arnold (1855) observed that patients with heart disease had a low vital capacity Bruns (1910) Bittorf & Forschbach (1910), Siebeck (1910) and Barr & Peters (1920) also observed that the vital capacity was reduced in heart disease but that the residual volume remained largely normal Peabody (1917) and Brittingham & White (1922) noted that the vital capacity diminished progressively with the degree of dyspnea and that it rose again when the pulmonary congestion was treated Binger s (1923) heart patients had a higher residual volume than normal but Lundsgaard (1923) found this to be true only of compensated cases his decompensated patients having a low residual volume Most of Hewlett s (1924) 900 patients with different types of heart trouble had a vital capacity below normal Richards Whitfield Arnott & Waterhouse (1951) confirmed Lundsgaard s observations regarding the residual volume Meakins & Christie (1929) found like Peabody, that the vital capacity fell with increasing dyspnea, and demonstrated a rise in the ratio between the residual volume and the total lung capacity in heart failure Altschule & Levine (1938) observed that diuretic therapy increased the vital capacity in heart patients and Abelman, Frank Gaensler & Cugell (1954) showed that the withdrawal of ascites increased the vital capacity In a study of orthopnea, Altschule, Zamcheck & Iglauer (1943) remarked that normal persons showed essentially the same changes in lung volumes on a change from the sitting to the supine position as did patients with heart disease West Bliss Wood & Richards (1953) stated that, in the absence of fluid retention, the lung volumes and maximum breathing capacity were not noticeably altered, even in marked pulmonary vascular engorgement Frank, Cugell Gaensler & Ellis (1953) reported that as the symptoms in mitral stenosis progressed, the vital capacity fell and the resting minute ventilation and respiratory rate rose slightly, while tidal volume fell that the residual volume was slightly higher than normal in early stages but fell with the onset of congestive failure and that there was no impairment in the distribution of inspired gas within the lungs

Christie & Meakins (1934) observed that a much larger change in intrapleural pressure was needed to change the lung volume by 20 per cent of the functional residual capacity in patients with pulmonary congestion than in normal subjects and that this difference diminished when the cardiac insufficiency was treated the intrapleural pressure was more posi

tive in their patients with pulmonary congestion than in their normal subjects Mead, Frank, Lindgren, Gaensler & Whittenberger (1953) noted a lowered dynamic compliance in cases of mitral stenosis, though the pressure in the pulmonary circulation was only slightly elevated in several of their cases Marshall, McIlroy & Christie (1954) discovered that the coefficient of elastic resistance was above normal in patients with mitral stenosis Brown, Fry & Ebert (1954) found a good correlation between static compliance and vital capacity in both normal subjects and heart patients They also noted a high total pulmonary resistance in heart patients and demonstrated by using mixtures of gases having other physical properties than air that this was referable to an elevated resistance to the flow of gas not to a change in tissue resistance They did not find that resistance to flow rose with a decrease in vital capacity

McIlroy & Christie (1954), and Marshall, Stone & Christie (1954) studying ventilation at rest and during exercise concluded that dyspnea is not conditioned by the mechanical work of breathing but by the force which has to be exerted on the lungs in order to increase ventilation Hayward & Knott (1955) observed that when patients with mitral stenosis exercised their high coefficient of elastic resistance rose even higher and that the values returned to normal after mitral valvulotomy

Saxton, Rabinowitz, Dexter & Haynes (1956) found no correlation between dynamic compliance and pulmonary vascular pressures pulmonary blood flow or pulmonary arteriolar resistance in heart patients in the supine position But during exercise 8 out of 9 patients showed a correlation between the degree of lowered compliance and the rise in pulmonary wedge pressure The compliance did not improve after operation for mitral stenosis, though the wedge pressure did so and the patients improved in clinical respects Nor did Frank, Lyons, Siebens & Nealon (1957) find any correlation between the drop in compliance and the rise in pulmonary arterial pressure but as a whole their heart patients showed a lower compliance than healthy subjects of the same height They also noted that the compliance was lowest in heart patients with signs of pulmonary edema and that compliance was positively correlated with both the vital capacity and the total lung capacity Pryor, Hickam, Sieker & Page (1957) and White, Butler & Donald (1958) noted a low compliance when the wedge pressure was high, but nor did they find any correlation between the increase in pressures and the drop in compliance Pryor *et al* (1957) reported that the compliance fell on exercise Verstraeten, van der Straeten & Pannier (1958) in turn got a negative correlation between the pressures and compliance in a series of patients with mitral stenosis Cherniack, Cuddy & Armstrong (1957) observed that a change from the sitting to the supine position caused a steep rise in the air flow resistance in heart patients but only a slight rise in normal subjects while the elastic resistance rose slightly in both groups

Nisell, Carlberger & Bevegård (1958), and Palmer, Gee, Mills & Bates (1963) showed that the compliance fell and the resistance rose with increasing disability in mitral stenosis Nisell *et al* (1958) also

noted that both the compliance and resistance fell when patients exercised. Recently Wood, Anthonisen and Macklem (1967) found that both static and dynamic compliance were lower than normal in 23 patients with mitral stenosis, but only at high lung volumes, not like in pulmonary fibrosis where compliance is decreased throughout vital capacity. They also found that airway resistance was about twice normal in mitral stenosis and that the slope of the maximal expiratory flow volume curve was depressed.

Ventilation in heart disease. Several authors have shown that congestive heart failure and mitral stenosis are characterized by above normal values for ventilation, both at rest and during exercise (Peabody, Wentworth & Barker, 1917, Barr & Peters 1920, Harrison, Turley, Jones & Calhoun 1931, Espersen, 1941, McIntosh, Sinnott, Milne & Reid, 1958, Stock & Kennedy, 1959). Gazetopoulos, Davies, Oliver & Deuchar (1966) found that patients with mitral valvular disease ventilated more than healthy subjects during exercise, and that the rise was significantly correlated with the wedge pressure.

Pulmonary congestion and edema. Pulmonary congestion or edema causes a drop in vital capacity and compliance (Christie 1935, Cherniack, Cuddy & Armstrong, 1957, Sharp, Griffith, Bunell & Greene 1958, Buhlmann, Behn & Schupp li, 1959, Sharp, Bunnell, Griffith & Greene 1961). Sharp *et al* (1961) reported that treatment of pulmonary edema increases compliance and conductance.

Studies of experimental pulmonary congestion induced in normal subjects by infusions of albumin (Pryor *et al*, 1957), Macrodex (Giuntini, Maseri & Bianchi, 1966), Rheomacrodex (Wilhelmsen & Varnauskas, 1967), or *g* suits (Bondurant, Hickam & Isley, 1957, Bondurant, Mead & Cook, 1960, Daly, Ross & Behnke, 1963) have shown a drop in compliance, and some studies have also revealed a rise in lung resistance. Wilhelmsen & Varnauskas found that the peripheral airway conductance decreased on the infusion of Rheomacrodex.

Animal experiments

The animal experiments done to examine the relationship between hemodynamics and respiratory mechanics have been concerned with the immediate effects. Von Basch (1891) contended that the increased volume of blood in the lungs in pulmonary congestion stiffened the parenchyma causing "Lungenstarre". Mack, Grossman & Katz (1947) showed in experiments on lung tissue and open chest dogs, that the distensibility of the lungs varied inversely with the amount of blood in the pulmonary vessels. Heyer, Holman & Shires (1948) found that infusing sodium salt solutions into dogs caused wider variations in pleural pressure but a drop in tidal volume. Borst, Berglund, Whittenberger, Mead, McGregor & Collier (1957) varied pulmonary blood flow and pressure independently in open chest dogs, and found that not until the pressure in the left atrium was raised to 30–40 cm H_2O did the compliance curve change. Hughes, May & Widdicombe (1958) stated that the compliance in perfused rabbit lungs fell about 25 per cent when the pulmonary arterial pressure was raised 40

mm Hg, and about the same amount in intact cat lungs when the pressure in the left atrium was raised 50 mm Hg Frank (1959) studying cat lung biopsies, observed that the recoiling force rose at high pulmonary volumes, was little affected in the medium volume range, and fell at low lung volumes—a phenomenon already noted in the classic studies of von Basch Frank concluded that there was probably a certain range over which the lungs and their blood vessels exerted a minimum of stress on each other Cook, Mead Schreiner Frank & Craig (1959) found that short periods of pulmonary congestion in dog lungs had little effect on the compliance but that after prolonged congestion the compliance dropped markedly On the other hand, they noted only a slight disorder in the overall compliance when the congested lungs were expanded over the region for tidal breathing They also remarked that the tissue showed distinct static hysteresis indicating that factors connected with surface tension were involved Levine, Mellins & Fishman (1965) used dogs to test different methods of differentiating between pulmonary engorgement and edema, including determination of the extravascular water space of the lungs with tritium They reported that the compliance fell moderately in pulmonary engorgement and greatly in pulmonary edema that the inspiratory lung resistance rose moderately in pulmonary engorgement and greatly in pulmonary edema, and that the end expiratory pressure at the functional residual capacity rose to less negative values in the first condition and to positive values in the second

Five groups of subjects were used for the present study (a) A control series consisting of 33 men between 18 and 70 (average age 43) and 39 women between 19 and 73 (average age 43) (b) A second control series consisting of another 20 women, aged between 18 and 71 (average age 45) studied for static compliance and peripheral airway conductance (c) A series of patients with mitral and/or aortic valvular disease, consisting of 20 men between 18 and 61 (average age 46) and 31 women between 32 and 63 (average age 47) This is henceforth called the main patient series (d) Ten women with mitral valvular disease aged between 29 and 71 (average age 52) studied for static compliance and peripheral airway conductance (e) One man and 6 women with mitral valvular disease aged between 27 and 70 (average age 54) studied before and after diuretic therapy Three of these are also included in series (d)

The complete breakdown of the five groups by age and sex was as seen at the top of the column on the right

Age	Control subjects Men	Control subjects Women		Patients with heart disease Men		Patients with heart disease Women		
	(a)	(a)	(b)	(c)	(e)	(c)	(d)	(e)
15–19	1	2	—	1	—	—	—	—
20–24	3	6	1	—	—	—	—	—
25–29	2	5	3	—	—	—	1	1
30–34	5	1	3	[illegible]	—	1	—	—
35–39	4	3	2	—	—	6	[illegible]	—
40–44	4	2	—	5	—	9	1	1
45–49	2	4	2	5	—	2	1	—
50–54	4	6	4	2	—	5	1	—
55–59	2	2	—	4	1	5	1	1
60–64	2	3	2	1	—	3	2	2
65–69	3	3	1	—	—	—	1	—
70–74	1	2	2	—	—	—	1	1
15–74	13	39	20	20	1	31	10	6

Extensive data were assembled on the previous and present medical history of each subject and for each subject a questionnaire on chronic bronchitis was filled in—i e a translation of the questionnaire made out by the British Medical Research Council's Committee on the aetiology of chronic bronchitis (1961) In addition a physical examination was made of the heart and lungs

Control series (a and b)

The two control series were made up of hospital employees who volunteered to be experimental subjects, and of patients admitted to two of the surgical departments for operation or analysis of their case whose underlying disease did not affect their heart or lungs, these were examined the first day they were admitted to the hospital

The requirements for inclusion in the control series were no morning cough or cough later in the day no expectoration no shortness of breath on hurrying on the level or walking up slight hills, and no wheezing normal observations on auscultation of the heart and lungs, and on fluoroscopy

None had a systolic blood pressure over 170 or a diastolic pressure over 100, and none were on antihypertensive medication None had severe varicose veins, or showed a tendency to edema in the legs All who had had any kind of inflammation were completely recovered from it when they were examined

Thirteen persons first considered as control subjects were excluded because of a history of respiratory disorder These 13 were between 33 and 72 (average age 50 years) None of the subjects meeting the original requirements for inclusion were excluded for other reasons

The control subjects were not chosen at random from the population and thus do not constitute a representative sample of the population They were collected so as to have a series to compare with the heart patients (who also included cases of hernia cholelithiasis and so on) The results from the control series were checked against those from 192 men aged 50 with healthy lungs These came from a large randomly selected series collected from an exhaustive survey of men aged 50 from Goteborg Out of the 973 men first yielded by the random selection data were collected for 855 The lung function of 399 of these 855 was tested and the aforementioned 192 were the ones of these 339 who met the requirements for a normal cardiac and respiratory history laid down for the other control series

Main patient series (c)

The main series which consisted of all the patients with disease of the mitral and/or aortic valve examined at the Sahlgrenska Hospital for diagnosed or suspected heart disease during the years 1963 to 1965 who could be catheterized, is not a random sample of patients with this disease but nor was it chosen in any systematic manner Table 1 in the appendix gives the main diagnoses and various clinical data in these cases The diagnoses were based on auscultation of the heart ECG s with chest leads roentgen examination of the heart and lungs in different projections with the size of the heart measured in the sitting and prone position and on the results of cardiac catheterization and in some cases angiocardiography

The functional disability was classed from I to IV in accordance with the criteria of the New York Heart Association (de Graff de la Chapelle Egglestone Kossmann, Lewy, Pardee & Schwedel 1942) The following shows the patients in the separate classes grouped according to grade of dyspnea in accordance with the criteria in the British questionnaire

Functional class	Grade of dyspnea			
	1	2	3	4
I	10			
II	14	18	1	
III		5	10	3
IV				4

The observations on the smoking habits among the controls in series (a) and (b) and the patients in series (c) to (e), divided by sex are seen at the top of the left column on the next page

The patients in series (c) were treated as one group in the various analyses despite the difference in their diagnoses 33 cases of mitral stenosis 5 of mitral insufficiency (1 of which also had aortic valvular disease), and 5 of uncomplicated

Tobacco cons g/day	Control subjects Men (a)	Control subjects Women (a)	(b)	Patients with heart disease Men (c)	(e)	Patients with heart disease Women (c)	(d)	(e)
0	13	25	13	8	—	21	6	4
	(39%)	(64%)		(40%)		(68%)		
x*	7	4	2	6	—	2	—	1
	(21%)	(10%)		(30%)		(6%)		
1–14	12	10	5	4	1	8	4	1
	(36%)	(26%)		(20%)		(26%)		
15–24	1	—	—	2	—	—	—	—
	(3%)			(10%)				
≥25	—	—	—	—	—	—	—	—
Total	33	39	20	20	1	31	10	6

* Ex smokers

aortic valvular disease (table 1 in appendix All numbered tables appear in the appendix) Mitral and aortic valvular disease differ clinically insofar as aortic stenosis is generally not attended by signs of pulmonary congestion at rest until the disease has been present a long time and long after the heart may have become considerably enlarged whereas mitral stenosis may be characterized by signs of pulmonary congestion at rest and even more on exercise and by only slight enlargement of the heart Three of the 5 patients with uncomplicated aortic valvular disease in the present study belonged to functional class I They had normal left atrial pressure at rest and may be considered slightly diseased Two cases with aortic stenosis belonged to functional class II and had elevated left atrial pressures (12 and 18 mm Hg) For the purposes of this study these two cases may be considered equivalent to the cases with mild mitral disease The hearts of the 5 patients were of about the same size as those of the patients with mitral valvular disease in the same functional classes

Eight patients (3 men and 5 women) had a history of cough and sputum for 3 months a year Symptoms of this kind are generally labelled chronic bronchitis, unless they come from another disease, such as local lesions in the lungs, specific generalized lung disease or primarily cardiovascular or renal disease, especially one leading to pulmonary edema (The British Medical Research Council's Committee on the aetiology of chronic bronchitis, 1965) Chronic bronchitis often leads to impaired ventilation and sometimes to very high resistance to airflow, and it is important to know in studies like the present whether respiratory symptoms are due to bronchitis or are secondary to the heart disease Three of the 8 patients had suffered from cough and expectoration long before they got their heart disease In 1 case, it was hard to say which came first, but it was probably the cough and expectoration In the other 4 cases the dyspnea, and probably other heart symptoms, began before the cough and expectoration For safety's sake all 8 cases were excluded when the effect of different factors on the lung mechanics was studied

Series studied for static compliance and peripheral airway conductance (d)

The 10 patients studied for static compliance and peripheral airway conductance were examined in the same way as the main series, but after analysis was started on the results from the main series Nine of these patients had uncomplicated mitral stenosis and one had mitral stenosis combined with mitral insufficiency Three had

a history pointing to chronic bronchitis (table 1) Only 4 underwent cardiac catheterization, otherwise they were all examined the same way as the other patients

Series studied for effect of diuretic therapy (e)

The 7 patients studied for the effect of diuretic therapy were also examined after the main series Six of them were admitted to the hospital for investigation, while the seventh was admitted for pulmonary edema The main diagnosis was mitral stenosis in 5 cases, mitral stenosis with slight mitral insufficiency in 1 and mitral insufficiency and systemic hypertension in the seventh (table 1) One subject belonged to functional class IV, 3 to class III and the others to class II None of them showed any clinical signs of pulmonary edema when they were examined or any roentgenographic signs of pleural transudate One patient had chronic bronchitis Only 3 underwent cardiac catheterization otherwise they were all examined the same way as the other patients

Roentgen examination

The roentgen findings noted in this study date from examinations done during one of the days immediately preceding the cardiac catheterization, after the patient had been put in as good condition as possible by digitalization and diuretic therapy. The roentgen pictures from each case were examined in one sequence all by the same roentgenologist according to a pre-arranged plan, and without the examiner knowing the diagnosis or about any of the hemodynamic or respiratory features in the case.

The size of the heart in the prone position was determined according to the method described by Larsson & Kjellberg (1948), as modified by Kjellberg, Rudhe & Sjöstrand (1949), and Kjellberg, Lonroth & Rudhe (1951). The frontal projection was exposed with the roentgen tube placed at an angle of 30 degrees cranialward with a transverse plane through the heart; the constant 0.53 was used consistently in the formula for the size of the heart.

The size of the heart in the standing position was determined according to the method of Jonsell (1939) with 150 cm between the focus and film and using a constant of 0.4 in the formula for the size of the heart. Both the prone and standing cardiac sizes were converted to volumes per square meter of body surface area, by dividing with the value for the body surface area reckoned from the DuBois nomogram (DuBois & DuBois, 1916).

The pulmonary roentgenograms were classified into five groups:

0 No evidence of vascular change.

1 Slight changes in the peripheral vessels in the lower lobes with reduced and/or irregular vascular markings and slight dilation of the perihilar pulmonary arteries.

2 Dilated middle sized vessels in the upper lobes and reduced and irregular vascular markings in the lower lobes and dilated perihilar pulmonary vessels.

3 More advanced stages of the foregoing changes and visible subpleural lines—Kerley B lines—and/or finely mottled vascular markings.

4 Dilated pulmonary veins and/or scattered, finely mottled, confluent parenchymal infiltrates indicative of acute venous congestion—pulmonary edema.

5 Impossible to judge state of vessels from roentgen pictures (2 patients).

None of the patients showed any large parenchymal densities or a large number of small densities.

Heart catheterization

Essentially the same procedure was used for the cardiac catheterization throughout—the procedure described by Forsberg (1964).

The catheterization was done in the morning without any premedication, with the patients in the fasting state, in the supine position. Some patients got a short acting barbiturate the night before. The

catheters were inserted under local anesthesia (1 per cent Carbocain, AB Bofors). One catheter was inserted into the pulmonary artery via a cubital vein, another into the left atrium via a transseptal puncture according to the method of Paulin & Varnauskas (1962), and a third either into the distal section of the aorta or into the brachial artery. After the patient had rested for 20 minutes during which no manipulations were done with catheters, the pressures in the pulmonary artery, the left atrium and a systemic artery were measured, and the cardiac output ($Qsyst$) and pulmonary blood volume ($Qpulm$) determined, and then the pressures measured again.

The cardiac output was determined by the dye dilution method. Five ml of bromsulphalein were injected into the pulmonary artery simultaneously with 3 or 5 ml of indocyanine green into the left atrium, or vice versa, and blood was collected from a systemic artery.

The pulmonary blood volume and mean pulmonary transit time ($MTTpulm$) were calculated according to the method described by Forsberg, viz.

$$Qpulm = \frac{MTTpulm\ (\text{sec}) \cdot Qsyst\ (L/\text{min}) \cdot 1000}{60}$$

The pulmonary vascular resistance (PVR) was determined from the following formula

$$PVR = \frac{\bar{P}_{PA} - \bar{P}_{LA}\ (\text{mm Hg})}{Q(L/\text{min})}$$

Here the same values for cardiac output as used for determination of the pulmonary blood volume were used.

Testing lung mechanics

Apparatus for measurement of volumes and flows

The respiratory volumes and flows were measured with a Servo Spirometer, model 130A (Med—Science Electronics, Ohio, Mo., U.S.A.).

The Servo Spirometer measures the flow and volume of gases with only minute loading effects on the source of power. The output of this instrument is controlled by a piston driven by a powerful motor which the relatively much weaker respiratory system is able to operate without getting loaded down. Geared to the piston is a potentiometer which measures the volume of gas and a tachometer which measures the flow of gas. The respiratory system moves the piston via a differential pressure transducer which monitors the pressure in the chamber of the Servo Spirometer and compares it with atmospheric. A deviation between these pressures of less than a millimeter of water produces an electrical signal from the pressure transducer. This signal is amplified and applied to motor driven clutches which move the piston until the pressure in the chamber returns to zero. Thus the pressure in the chamber is maintained at an approximately atmospheric level, and breathing into the chamber meets with very little resistance.

The tubing for this instrument, including the mouthpiece, is about 16 cm long and 41 mm wide at its smallest diameter. For measurements of static compliance we add on a tube with an inner diameter of 22 mm and a manually operated shutter valve. The instrument has no carbon dioxide absorber and no extra oxygen supply. The chamber of the

Servo Spirometer is equipped with a thermometer

If the pressure in the chamber of the Servo Spirometer is maintained at an atmospheric level, the piston will move in proportion to the amount of inflowing gas But the pressure in the chamber has to deviate for a certain length of time in order to provide the error signal which commands the piston to move This causes a slight error in the volume data obtained from the potentiometer The voltage produced from the pressure transducer is a measure of this volume error Adding this signal to the potentiometer signal thus yields the correct volume data

The signal obtained from the tachometer is subject to a similar error and correction is made by adding the derived pressure error to the tachometer signal

The volume calibration is done by a ten turn potentiometer adjusted so that it gives one liter for one turn The Servo Spirometer has a capacity of ten liters

The potentiometer controlling the flow calibration can be adjusted with a one liter per second flow source connected to the Servo Spirometer

Testing the Servo Spirometer Before the Servo Spirometer was used for this study it was tested for leakage between the cylinder and the piston sensitivity of the pressure transducer linearity of the volume recording linearity of the flow recording reaction time of the circuits, and simultaneity of the flow and volume signals

If the chamber of the Servo Spirometer is closed inserting 50 ml of air into it should produce a 5 volt change over a special measuring point If the chamber leaks or if the rubber membrane covering the piston is too slack, inserting the air will have less effect on this voltage or one that rapidly disappears It turned out that we had to change the packing between the cylinder and the piston in our instrument because of this kind of leakage After we had done this the proper 5 volt change lasting a sufficiently long time was noted, the voltage amounting to 4 7 volts 20 seconds after the air was injected, indicating that for practical purposes the chamber could be considered air tight After this we tested the membrane once a month, and exchanged it for a new one whenever necessary—as it turned out at intervals of four to six months

Testing the sensitivity of the pressure transducer by attaching it to a water manometer showed that differences in pressure relative to atmospheric of one millimeter of water gave signals to the spirometer causing the piston to move

The accuracy of the measurements of volume was tested by measuring the internal diameter of the Servo Spirometer and calculating the movements of the piston in liters The circuit of the volume potentiometer was corrected so that one turn corresponded to one liter for all conceivable positions of the piston After this a calibrated strip of tape was attached to the chamber of the spirometer, so that it was easy to do the geometric calculation before each subject was tested and at the same time as the spirometer was electrically calibrated

The electrical calibration of the flow circuit was done in the simple way prescribed by the manufacturers of the spirometer before each subject was tested If the electronic system was correctly ad

justed the electrical calibration always produced a signal corresponding to one liter of air flow We test this detail about once a year and have never noted any changes in the amplitudes of the flow circuit The manufactures of the Servo Spirometer lock the potentiometer controlling the flow calibration

The following was done to test the exactitude of the flow data A constant flow of air was led to the chamber of the spirometer The volume and flow were registered on the recorder running at high speeds—up to four inches a second—and the data for the constant flow compared with the slope of the recorded volume The following shows the results of this testing

Flow calculated from volume and time *LPS*	Flow values from the flow channel *LPS*
0 47	0 47
1 07	1 0
1 67	1 66
2 68	2 60
5 49	5 45
8 01	8 18
11 6	11 9
13 8	14 4

As seen the differences were insignificant and stemmed partly at any rate from the difficulty in measuring with an exactitude under 0 5 mm

McCall Hyatt Noble & Fry (1957) showed that there was a significant amplitude content defined as 5 per cent or more of the fundamental out to about 4 Hz in ordinary tidal breathing Hence a spirometer for registering this type of breathing should have a quick enough reaction time to be able to reproduce frequencies up to this magnitude without much damping The Servo Spirometer proved to be able to reproduce frequencies up to 10 Hz

The volume and flow circuits being of different construction in the Servo Spirometer, the difference in time between the two kinds of signals was checked with a generator producing square sine and triangular waves This showed that the flow signal came 6, 12 10 and 6 msec after the volume signal for the attenuator settings 1 2, 5 and 10 respectively It being impossible to change the time constants in the flow circuit without heightening the noise the time constant of the volume circuit was increased instead After these corrections, the total delay for both circuits amounted to 30 msec and the difference between the delay in the flow circuit at different attenuator settings fell to less than 1 msec

Measurement of esophageal pressure

Measurement of the intra esophageal pressure has proved to be a reliable and useful way of estimating pleural pressure In this study the esophageal pressures were measured by the method described by Milic Emili Mead Turner & Glauser (1964a) using a 10 cm long rubber balloon with thin walls (about 0 9 mm thick) and a perimeter of about 35 mm The balloon was placed over the end of a polyethylene catheter (inner diameter 1 57 mm outer diameter 2 08 mm length 80 cm) perforated at this end with about ten small holes The rubber balloons were made in the laboratory from a liquid rubber solution. Before every measurement the balloon catheter unit was inflated in water to see that it did not leak

The balloon catheter unit was connected to a differential pressure transducer of the variable inductance type (Elema Schönander, EMT 490 Bd, range 0 to ±30 mm Hg, volume displacement 0 003 ml per 100 mm Hg) and the pressure transducer to an amplifier (Elema Schönander EMT 460) Because of the small volume displacement coefficient of the catheter manometer unit, the volume of the balloon changes only a little on a change in pressure The pressure was always measured in the area of linear response of the system The frequency response for the whole system, including the esophagus balloon and recorder, was tested with square waves (balloon rupturing) and calculated according to the formula $B \cdot T = 0.35$, with B equal to the frequency response, T the time needed for changing the response from 10 to 90 per cent of the full scale response According to this B amounted to 23 Hz

The balloon catheter unit was inserted into the esophagus through a nostril and the end pushed down 40 cm from the nostril except when the subjects were very short when it was not pushed down so far or very tall when it was pushed down 43 to 45 cm

It is possible to ensure that the balloon is in the esophagus by inserting the balloon down to the stomach where positive pressures obtain then drawing it back slowly until negative pressures are registered showing that the balloon has entered the esophagus and then drawing it back another 10 cm Comparison between this method and the one used in this study showed that the two gave practically the same results

The balloon catheter unit was connected to a system consisting of a water manometer and syringes for calibrating the system Before each measurement, about 5 ml of air were introduced into the balloon to distend its walls evenly after which the air was withdrawn until only 0 2 ml was left

When small mouthpieces (22 mm inner diameter) were used, the transpulmonary pressure was calculated from the differences between the esophageal pressure and the pressure on the inner surface of the mouthpiece When larger mouthpieces (41 mm inner diameter) were used the difference between the pressure in the oral cavity and the atmospheric pressure was generally impossible to measure amounting to about one mm H_2O at the most on normal respiration and it was therefore concluded that it was not necessary to measure oral pressure routinely The small difference in pressure can be ascribed to the great sensitivity of the Servo Spirometer and the small resistance to flow in the connecting tubing

Pressure irregularities caused by the action of the heart were smoothed out by drawing the mean curves for the pressure In 3 cases of heart disease this method proved to be unreliable and they were therefore excluded from the study

Comments Simultaneous measurement of the esophageal and pleural pressure in the sitting position have shown that the two correspond acceptably (Fry Stead Ebert Lubin & Wells 1952 Attinger Monroe & Segal 1956 Butler White & Arnott, 1957 Mead & Gaensler 1959) Mead & Gaensler (1959) noted that the flow resistive pressures corresponded better than the elastic components of the

pressure For practical reasons, and because the pleural and esophageal pressure differ so little at least with the subject sitting, most studies on pulmonary mechanics have used the esophageal pressure

Lately, however, it has been noted that *the amount of air in the balloon its position in the esophagus and its size* all make a difference to the values obtained Milic Emili *et al* (1964*a*) showed that the esophageal pressure rose with the volume of the balloon at all lung volumes, but not uniformly, the volume of the balloon having most effect at both extremes of the *VC* They showed that errors of measurement could be eliminated by repeating measurements with different balloon volumes and extrapolating the esophageal pressure to the zero balloon volume, or by measuring with extremely small balloon volumes In their experience a volume of 0 2 ml gave satisfactory measurements with balloons 10 cm long with a perimeter of 35 mm Milic Emili, Mead & Turner (1964*b*) demonstrated the significance of the position and length of the balloon. Thus they noted that in approximately the upper third of the esophagus changes in the mouth pressure position of the head and external pressure on the trachea produced variations in pressure which were not related to variations in the pleural pressure but were probably the result of traction on and/or compression of the esophagus by the trachea The pressure values they obtained from the *lower two thirds of the esophagus* were not subject to these errors They noted likewise that in the lower third of the esophagus the pressures varied greatly from spot to spot and with the position of the body From all this they concluded that the esophageal pressure should be measured in the middle third of the esophagus and pointed out that with the longer balloons (15–20 cm) previously used, it was hard to eliminate the effects from the upper part of the esophagus *unless a part of the balloon lay in the stomach*

Another source of error not yet fully evaluated, is pressure exerted by other nearby organs on the esophagus—the heart for example The heart might cause considerable errors of this kind when the subjects are in the supine position but it should have less effect when they are examined in the sitting position Frank *et al* (1957) tried to determine the effect of the heart by comparing the values for static compliance obtained with an esophageal balloon in the lower part of the esophagus and in the middle or upper part but they found no difference between the two situations Wood *et al* (1967) made a careful study of the three possible sources of artifact (1) increased esophageal elastance because of the heart pressing on the esophagus (2) the heart acting as a mass load on the esophagus (3) the heart acting as a mass load pressing on the esophagus to different degrees at different lung volumes Their studies could exclude the first and the third artifact They could not exclude the second one—the mass load effect—but the authors concluded that it should only invalidate the use of esophageal pressure for estimating the absolute values of pleural pressure

In the present study the level of absolute pressure in the esophagus at functional residual capacity (*FRC*) was significantly higher in the women with heart disease

(−4.3) than in the women without (−7.4)—$P < 0.001$. The size of the heart and the esophageal pressure at *FRC* were not correlated in the heart patients. In no series was the esophageal pressure at *FRC* related to the expiratory reserve volume (*ERV*).

Christie & Meakins (1934) made a corresponding observation, that the pleural pressure in 5 heart patients was higher than in normal subjects.

Recording instruments

The volume, flow and pressure were recorded on a Honeywell Visicorder model 1012, and with a Tektronix Oscilloscope, model 530, equipped with a camera.

The Visicorder is an optical recorder with mirror galvanometers situated 30 cm from the recording paper, which is sensitive to ultraviolet light. Paper speeds from 0.1 to 160 inches a second can be used. Time lines can be marked on the recording paper at intervals of 10.0, 1.0, 0.1, 0.01 and 0.001 seconds. The lines are produced by means of a gas discharge flash tube synchronized to the sine waves in the source of current, resulting in exact time marking. The galvanometers are characterized by a maximum peak deflection with 2 per cent linearity amounting to 20 cm, and flat frequency responses of 0–60 and 0–120 Hz.

The oscilloscope was connected parallelly with the Visicorder to the Servo Spirometer and the pressure manometer. The different channels could be registered at right angles to one another in the following way, with the vertical signal given first: flow-pressure, flow-volume and pressure-volume. By chopping the signals it was possible to see the two latter combinations at the same time.

The oscilloscope was used together with a ten turn potentiometer unit to eliminate electrically the elastic component from the esophageal pressure on the pressure-flow curve of the oscilloscope to get a curve for the pulmonary resistance according to the method of Mead & Whittenberger (1953). The resistance pressure obtained this way was fed to the Visicorder, making it easy to obtain this part of the esophageal pressure as a function of the time. In this way all the measurements could be seen at one time on the oscilloscope, whose pictures were photographed with a polaroid camera.

All the recording was done simultaneously with the Visicorder. All the calculations were done on the Visicorder records, where the measurement error was least. Throughout this study, about the following trace deflections were used.

Volume	1 *L*	= 40 mm
Flow	1 *LPS*	= 30 or 7.5 mm
Esophageal pressure	10 cm H_2O	= 40 or 16 or 8 mm
Resistive pressure	10 cm H_2O	= 50 mm

In some cases the degrees of amplification had to be changed so as to be able to keep within the linear range for the different measuring instruments (Servo Spirometer, pressure manometer and Visicorder).

The data for flow and volume were corrected for temperature and expressed at body temperature and pressure saturated with water vapor (*BTPS*).

Lung volumes

Vital capacity and forced expiratory volume I did all the testing myself with the subjects in the sitting position. They were informed in full about all the tests they were to undergo and it was checked that they cooperated by watching the curves for flows and volumes on the oscilloscope screen and the pressures on the manometer

All the subjects were requested to perform at least three slow vital and at least three forced vital capacity manoeuvres and the maximum values for VC and forced expiratory volume per second (FEV_1) were calculated from the resulting curves Measurements on the forced expiration tracing were begun from the first point of deflection The paper was generally run at a speed of 1 0 inch per second The FEV_1 in per cent of VC ($FEV_1\%$) was calculated from the maximum values for FEV_1 and VC

Comments Measurements of VC show good reproducibility Miller Johnson Jr & Wu (1959) stated that a maximum difference of 100 ml is permissible between double determinations and Kory Callahan Boren & Syner (1961) allowed a 5 per cent difference The means for the highest values for VC in 20 randomly selected controls from the present study differed from the means for the next highest values by 0 06 L or 1 4 per cent and in 20 randomly selected heart patients by 0 06 L or 1 9 per cent These values correspond with those from other studies of normal subjects—for example with the 1 3 per cent in the normal series of Birath Kjellmer & Sandqvist (1963)

Comparative studies have indicated that FEV_1 is a good indirect measure of the airway resistance and that it is a better measure than the maximum voluntary ventilation (MVV) (Attinger Goldstein & Segal 1958 Ehrner, 1960 Johnson, Jr Miller & Wu 1962)

The means for the highest values for FEV_1 differed from the means for the next highest values by 0 04 L (1 2 per cent) and 0 15 L (6 0 per cent) in the aforementioned 20 controls and 20 patients from the present series According to Miller *et al* (1959) the maximum values can be reproduced with a difference of less than 50 ml Ehrner (1960) found a relative experimental error of 4 8 per cent in a group of normal subjects and patients and Birath *et al* (1963) observed that the highest values of normal subjects never differed by more than 4 per cent from the next highest values on the same occasion Ringqvist (1966) noted that the relative error of a single determination in his series of normal subjects amounted to 3 6 to 4 9 per cent

Functional residual capacity and residual volume The FRC and RV were measured directly before or after the tests previously described with the subjects sitting (in a chair tilted about 15 degrees backwards) according to the method described by Meenely & Kaltreider (1949) as modified by Holmgren (1954) While the helium was mixing with the air in the lungs the subjects made three or four maximum expirations After the concentration of helium in the lungs had reached a constant level they made one more maximum expiration and finally a maximum inspiration whereupon the testing was ended

The FRC was taken as the sum of the ERV (at the time the compliance and conductance were measured) and the RV

Comments Grimby & Soderholm (1963) found that the error of a single measurement of the FRC with the same apparatus amounted to 7 5 per cent, a value much like that found by other authors (Gilson & Hugh Jones 1949 Holmgren 1954 Ehrner, 1960 Ringqvist, 1966) The values for normal subjects obtained with this protracted dilution method agree well with those obtained with a body plethysmograph (Tierney & Nadel, 1962) but the helium method underestimates the volume of gas in poorly ventilated parts of the lungs

The position of the body affects the lung volumes particularly the ERV which increases on a change from the supine to the sitting position (Blair & Hickam 1955 Craig, 1960 Moreno & Lyons 1961 Hanson Tabakin & Caldwell 1962) Craig (1960) also showed that only slight shifting of the body position such as on moving the elbows could lead to changes in the ERV

In the present study the lung mechanics were studied with the subjects sitting in a slightly different type of chair than for the RV Regression analysis of the values obtained for ERV with the helium method and on the other examinations gave the following equations for the control series $ERV_{HE} = -0\ 68 \quad ERV_{ME} + 0\ 31$, $R = 0\ 87$ $RSD = 0\ 26$ For the heart series the equation was $ERV_{HE} = +0\ 57 \quad ERV_{ME} + 0\ 54$ $R = 0\ 57$ $RSD = 0\ 35$

Maximum expiratory flows

Flow volume curves were registered according to the method of Hyatt *et al* (1958) for all the subjects The values for maximum expiratory flow at 50 per cent of the VC ($MEF50\%$) and 25 per cent from the residual volume ($MEF25\%$) were measured to get a numerical value for the flow volume relationship To get a numerical value for the slope of the flow volume curve the difference in flow between 50 and 25 per cent of VC was determined, as well as the difference between 25 per cent and maximum expiration (zero flow), and the two values divided by the difference in volume ($=1/4VC$)

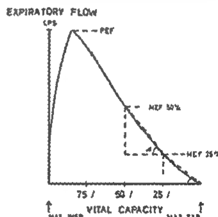


Fig 1 Example of maximum expiratory flow volume curve explaining symbols used

The flow volume curves were then assumed to be straight lines between the points making the expressions equivalent to the tangent angle with the horizontal axis (fig 1) where the tangent $\alpha = 4 \quad MEF25\%/VC$ and the tangent $\beta = 4(MEF50\% - MEF25\%)/VC$ In addition the value for the tangent β divided by the tangent α was calculated to get a measure for the difference in slope in different parts of the curve

Comments The relationship between the maximum flow and the lung volume was studied by Fry Ebert Stead & Brown (1954) Hyatt *et al* (1958) and Dayman (1958) Hyatt *et al* (1958) demonstrated that the ratio was remarkably reproducible over the lower range of the *VC* They found that the dynamic compression of the airways increased as the lung volume fell and that the flow ceased to increase beyond a certain degree of effort They noted that the value for the maximum flow depended on the lung volume and found that the flow volume curves were of different appearance in normal subjects and persons with heart disease and obstructive lung disease

Later Fry (19 9) showed with a simple lung model that the relationship between maximum flow and lung volume was independent of the resistance of the upper extrathoracic airways that it depended on the resting dimensions and physical properties of the intrathoracic airways where most of the lesions accompanying lung disease are situated

Fry & Hyatt (1960) demonstrated that increase in the external airway resistance did not affect the appearance of the lower part of the flow volume curve in normal persons The effect of adding an external resistance on the results in 23 men from the 50-year old series was

Schilder Roberts & Fry (1963) demonstrated that the flow volume curves reacted to changes in density and viscosity of the inspired gas the lower part reacting most strongly to a change in viscosity and the upper part most strongly to a change in the density These results agree with the theories developed by Fry (1959)

Several other authors have studied the flow volume curves notably Burger (1959) Bartlett (1961) Branscomb (1962) Sobol & Emirgil (1964) Hyatt (1965) and Wood *et al* (1967) Sobol & Emirgil (1963) analyzing the reproducibility of different parts of the curves from 200 persons came to the conclusion that other parts of the curves were just as reproducible as the last parts

In the present study the means of the highest values for peak expiratory flow (*PEF*) differed from the means for the next highest on the same occasion by 0 [illegible] *LPS* (9 4 per cent) and 0 53 *LPS* (10 5 per cent) in controls and patients with heart disease respectively The corresponding values for *MEF*50% were 0 [illegible] *LPS* (12 1 per cent) and 0 40 *LPS* (12 [illegible] per cent) and for *MEF*25% 0 [illegible] *LPS* (12 [illegible] per cent) and 0 16 *LPS* (12 0 per cent)

The compressibility of the air in the lungs has a noticeable effect on the [illegible] ance of the flow volume curve [illegible]

	Control period		With external resistance		Difference	[illegible]
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	%	
VC L	4 59	0 16	4 [illegible]0	0 14	− [illegible]0	[illegible]
*FEV*1 *L*	3 46	0 13	2 35	0 13	−3[illegible]	
*FEV*1%	[illegible]	1 [illegible]	53 [illegible]	3 1	[illegible]	
PEF LPS	6 99	0 [illegible]9	3 00	0 [illegible]	[illegible]	
*MEF*50% *LPS*	4 07	0 26	2 35	0 16	[illegible]	
*MEF*25% *LPS*	1 [illegible]9	0 13	1 30	0 13	[illegible]	

in forced expiration (Rahn, Otis, Chadwick & Fenn, 1946, Johnson 1962) Jaeger & Otis (1964) published an exhaustive analysis of its importance Because the alveolar gases are compressed and expanded during every phase of breathing, the variations in volume measured at the mouth are smaller than the ones measured with a body plethysmograph The differences between the two sites are small in normal persons, but they may be large in persons with obstructive lung disease Ingram & Schilder (1966) studied the effect of gas compression on the flow volume curves

In the present study, corrections were made for gas compression from the values for gas volume static recoil pressure and the esophageal pressure during forced expiration in the 30 cases studied for static compliance and peripheral airway conductance

The flow volume curves were drawn with the flow on the ordinate and the volume on the abscissa The slope of the curve at any point can be expressed as the tangent for the angle with the abscissa i e the ordinate value divided by the abscissa value or

$$\frac{\dot{V}}{V} = \frac{\text{volume/unit time}}{\text{volume}} = \frac{1}{\text{unit time}}$$

In a system of bronchi and alveoli the resistance times the compliance ($R \cdot C$) is equal to the time constant of the system in question McIlroy Tierney & Nadel (1963) used this relationship in relaxed expirations together with an extra known resistance for measuring the compliance and resistance of the lungs and thorax.

Different parts of the lungs are emptied at different volumes during maximum expiration Thus the parts with high time constants contribute to a larger part of the total flow at the end of expiration after the better ventilated areas have emptied One can think in terms of a spectrum of different time constants in the lungs giving the flow volume a curvilinear course sloping more gradually towards the point of maximum expiration, i e at the part covering high time constants A leveling off of this kind is not seen in young healthy persons in whom the time constants are relatively uniform throughout the lung, but it is seen in older subjects (Turner, 1963 Mead *et al*, 1967)

Sometimes extrapulmonary factors affect the shape of the curve Thus, in some young persons the flow drops sharply from a relatively high flow (about 1 *LPS*) down to zero It is possible to produce further decrements of lung volume in these young subjects by external pressure on the thorax (Leath & Mead, 1967)

Peripheral airway conductance

The peripheral airway conductance was measured according to Mead *et al* (1967) in small series of normal subjects and patients with mitral valvular disease

In this study the static compliance of the lungs and with this their elastic recoil—*Pst(l)*—throughout the whole range of the *VC* was determined whereafter flow volume curves were produced The peripheral airway conductance was then calculated as the maximum flow ($\dot{V}\,max$) divided by the *Pst(l)* for the lung volume in question

Comments Mead *et al* (1967) said that the shape of the flow volume curve could be explained by the waterfall principle for compressible tubes formulated by Permutt, Bromberger Barnea & Bane (1962) reasoning as follows The maximum flow at a given lung volume is proportional to the $Pst(l)$ at the lung volume in question $Pst(l)$ produces flow in the airways between the alveoli and the points where the pressure has fallen from the pressure in the alveoli ($Palv$) by a value exactly corresponding to $Pst(l)$ But as $Palv$ minus $Pst(l)$ is equal to the pleural pressure (Ppl) the airway pressure at these points is exactly the same as Ppl If these points which they called equal pressure points (EPP) are situated in the intrathoracic and intrapulmonary airways the transmural pressure over the airways amounts to zero and any airway obstruction occurring during maximum expiration must come between these points and the mouth The flow is then determined by the resistance between the alveoli and equal pressure points They called the resistance of these segments of bronchi the peripheral airway or upstream resistance (Rus) and calculated as follows

$$Rus = \frac{Pst(l)}{\dot{V}max}$$

Both $\dot{V}max$ and $Pst(l)$ are constant at every lung volume Therefore Rus must also be constant

Lung compliance and conductance

The effective or dynamic compliance—$Cdyn(l)$—was measured after a maximum inspiration followed by a maximum expiration in order to determine the ERV Five complete undisturbed respiratory cycles were used for calculating this variable, the first two or three tidal breaths being excluded Major changes in the FRC level (more than 50 per cent of the tidal volume) were not tolerated The compliance was calculated as tidal volume divided by corresponding pressure difference at zero flow The average of ten determinations was used to represent each subject

To get the FRC associated with the compliance the ERV was calculated and its value added to the RV (helium method)

The static compliance was determined in 30 cases The static recoil pressure (Pst) was measured with the tube between the mouthpiece and spirometer shut off for 1–3 seconds by means of a manually operated shutter valve inserted in the tube Curves were then drawn for the expiration over the whole VC In some cases pressure artifacts arose near maximum expiration and the true pressures were estimated by drawing the curves from points free of artifacts to zero pressure at RV At least three curves were recorded for each subject and the one showing the greatest negative pressure at maximum inspiration and the fewest artifacts was used

The total pulmonary conductance was computed as the flow divided by the resistance pressure ($Pres$) the $Pres$ being measured at four different places on each cycle at the beginning and end of inspiration and expiration where flow was exactly 0.5 liters per second The mean conductance during the inspiration—$Gi(l)$—and expiration—$Ge(l)$—of five breaths was calculated but as a rule only the inspiratory conductance was used for further calculations in this study

Comments In reality no one value can give a proper idea of the elasticity of a given lung It is easier to see this from the static pressure volume curve for the whole *VC* Most researchers have only used the slope of the curve in the region for the tidal volume, in which the function is approximately linear (Butler *et al*, 1957) whereas the curve as a whole is sigmoid shaped

Several studies have reported that normally *Cdyn(l)* is usually independent of the respiratory rate up to about 60 to 90 breaths a minute (Butler *et al*, 1957 Cherniack, 1956 Defares & Donleben, 1960 Mead & Whittenberger 1953 Mead, Lindgren & Gaensler 1955 Otis McKerrow Bartlett Mead, McIlroy Selverstone & Radford 1956 von Rau, Behn, Gebhardt Rossier & Buhlmann 1957) Later Mills Cumming & Harris (1963) found that the compliance fell with an increase in respiratory rate at three different respiratory levels in eleven normal adults aged between 18 and 38 Pierce & Ebert (1958) and Cohn & Donoso (1963) made similar observations in older healthy subjects but Frank Mead & Ferris (1957) did not observe any drop in a group of elderly persons with a normal airway resistance though they did in a group with a slightly raised airway resistance

According to Otis *et al* (1957) independence of the respiratory rate on *Cdyn(l)* must mean equal time constants throughout the lung On the other hand Defares & Donleben s (1960) experiments showed that a rate dependent compliance is not necessarily a sign of uneven ventilation

For the present study of *Cdyn(l)* the subjects were asked to breathe as slowly as they could The male controls breathed at an average rate of 17 6 breaths/min (s_x=4 12, range 11–25) the female controls at 18 3 (s_x=5 4 range 6–31) and the whole series of heart patients at 14 0 (s_x=5 7 range 6–20) The respiratory rate had no effect on the compliance in the control subjects

The volume history of the lungs before the compliance is measured affects the values obtained Thus Ferris & Pollard (1960) showed that the compliance rose after deep breaths In their normal subjects this effect reached its peak after two deep breaths but in their patients it went on rising with further deep breaths Mead & Collier (1959) noted that forced inflations of the lungs increased the compliance while forced deflations lowered it

In the present study the compliance was always measured after maximum expiration To see if the compliance changed during the measuring period (at least five breaths) the values for the first and last breathing cycle were compared in 20 randomly chosen subjects from the control group and 20 from the group with heart disease This showed no significant difference in the compliance during this period (+4 38 per cent) The tidal volume did not change during the course of the measuring period

The standard error of a single determination of *Cdyn(l)* was calculated from the 40 randomly chosen control subjects and heart patients and amounted to 0 007 *L*/cm H_2O (3 6 per cent) and 0 005 *L*/cm H_2O (3 6 per cent), respectively Frank Mead Siebens & Storey (1956) measuring *Cst(l)* of 42 healthy subjects twice in succession with the esophageal balloon fixed in one position got a standard deviation of ±0 015 *L*/cm H_2O ex

amining 10 persons at intervals of 3 to 12 weeks they got a standard deviation about the mean for the two measurements of 0 019 *L* cm H_2O Marshall (1965) meas uring *Cdyn(l)* of two persons ten times in the space of 17 days calculated that the coefficient of variation between the meas urements on different days amounted to ±10 2 per cent for each subject

The figures for conductance obtained in the present study represent both the air way and tissue conductance usually com bined under the name total pulmonary conductance Several have attempted to determine how much the tissue resistance contributes to the total resistance A num ber have tried to do so by changing the physical properties of the gas inspired (e g Bayliss & Robertson 1939 Fry *et al* 1954 McIlroy Mead Selverstone & Rad ford 1955) McIlroy *et al* (1955) calculated by this method that it accounted for about 30 to 40 per cent Using the body plethys mographic method Marshall & DuBois (1956) reckoned that it answered for 18 per cent of the total pulmonary resistance Ferris Mead & Opie (1964) got a figure of 10 5 per cent while Jaeger & Otis (1964 b) found it to be 28 per cent

Some observations indicate that the tissue resistance drops when the respira tory rate rises (Mount 1955 Setnikar & Meschia 1952) if so it would be hard to determine the part played by tissue resist ance in the whole resistance Whether disease affecting the lungs—for example mitral stenosis—makes a difference to the relative contribution of tissue resistance is not known but Brown *et al* (1954) concluded after studies with gases of different viscosity that the increase in pulmonary resistance in 7 heart patients was almost solely attributable to an in crease in re istance against gas flow

Because of the sigmoid shape of the pressure flow curve one can only compare values for conductance (resistance) meas ured at the same rate of flow A flow of 0 5 *LPS* was chosen in the present study as this rate generally lies within the recti linear section of the pressure flow curve corresponds to relatively slow breathing and can be managed by persons with obstructive lung disease

Nadel & Tierney (1961) observed that a deep breath did not affect the airway resistance measured with a body ple thysmograph in normal persons but re duced it when the bronchi were con stricted In the present study all the measurements were done after maximum expiration The *Gt(l)* at the beginning and end of the period during which it was measured did not differ significantly in 40 randomly chosen subjects

The error of a single measurement of the resistance calculated from the same randomly chosen persons from the two series amounted to 0 29 cm H_2O/LPS (18 7 per cent) and 0 33 cm H_2O/LPS (15 4 per cent) respectively Marshall (1957) reported that the coefficient of vari ation of 10 measurements during a space of 17 days in 2 subjects amounted to 13 1 and 12 9 per cent respectively

Statistical methods

A Facit EDB3 computer was used for the multiple regression analysis and for cer tain forms of sorting calculation of means and other operations For certain other calculations and for a number of bivariate and trivariate regression analyses an Oli vetti Programma 101 computer was used

The conventional statistical methods were used for calculating the mean (x) and the standard error of the mean (s_x). The difference between means was denoted with $\bar{d}$ and the standard error of this difference with $s_{\bar{d}}$ (see, for example, Snedecor 1965). The mean value and its standard error are given for every computation.

The formula $\sqrt{\frac{\Sigma d_i^2}{2n}}$ was used to calculate the standard error of a single determination in a series of determinations whose mean difference was not significant (Dahlberg 1948).

For testing the reproducibility of VC, FEV_1, PEF, $MEF50\%$ and $MEF25\%$, the mean of the highest values in the series was calculated and compared with the mean for the next highest values.

The multiple regression analysis was done on the assumption of linear relationships, except that the independent variable height was sometimes raised to the third power (height³). No curvilinear regression analysis was done. This might have revealed stronger correlations between the variables compared in the last part of this study.

The independent variables were chosen on the basis of previously known conditions or biologically conceivable relationships. The analysis was generally started with one or two independent variables and whenever any significant relationships emerged continued with the inclusion of others. The effect of adding an independent variable was judged from its effect on the determination coefficient (R^2). (With an R^2 of 1, the whole relationship is due to the independent variables in question; if equal to 0.50, 50 per cent is due to the independent variables, and so on.) The significance of the independent variable could also be seen from the value for the residual standard deviation (RSD).

All the hypotheses on relationships were tested using a significance level of 5 per cent. Only relationships reaching this significance level are included in the tables showing regression equations (apart from occasional instances given in parentheses). The regression coefficients (b) and the standard errors for these (s_b) are given for all the regressions.

When the means of different groups of heart patients were compared with the means predicted by regression analysis of data from the control series, the standard error was corrected according to the following formula, consideration being given to differences between the patients and controls in means for age (x), height ( ), weight (y), and so on.

$$s^2_{calculated} = \frac{(RSD^2)}{n} + s^2_{b_x}(x_{patients} - x_{controls})^2 + s^2_{b_z}(\;_{patients} - \;_{controls})^2 + \text{etc}$$

$$t = \frac{y_{predict} - y_{observed}}{\sqrt{s^2_{calculated} + s^2_{y\,observed}}}$$

Several have studied how age height and weight affect the lung volumes for healthy men and some also for women Cotes, Rossiter, Higgins & Gibson (1966) have assembled the results for men These results agree well from series to series even between series of randomly selected subjects and for example subjects in certain occupations

The present study used several other tests than the ones these authors took into account and as normal values for these are lacking a special control series had to be assembled for this study Statistical consideration of the number needed for multiple regression analysis and the other operations used showed that about 30 persons of either sex would be sufficient for comparison with the heart series Whenever possible the results from the control series were also compared with control data from the literature Some of the results in the present series could also be compared with the results from a randomly selected series of men aged 50 (Wilhelmsen Tibblin & Eld 1968) 102 of whom had no cardiopulmonary symptoms This series of randomly selected subjects enabled a more exact regression analysis with the other anthropometric variables

The connection between different anthropometric variables and static pulmonary volumes was tested by linear regression analysis The testing was first done with all the variables age height height³ weight and body surface area (*BSA*) The relationship with the lung volumes such as the total lung capacity vital capacity and functional residual capacity was also tested It was determined whether any of the independent variables were correlated It turned out that height and weight were positively correlated in the men but not in the women

Some authors have said that different lung volumes might be better correlated with height as a logarithmic function or raised to the third power and this has been shown to be true of children (Morse Schultz & Cassels 1952 Engstrom Karlberg & Kraepelin 1956 Helliesen Cook Friedlander & Agathon 1958 Cook & Hamann 1961 Bjure 1963)

Results of tests of lung mechanics

Table 2 gives the means standard errors and ranges of the values obtained and table 3 the results of multiple regression analysis

The men and women differed in height averaging 176 8±1 2 cm ($\bar{x}$ and $s_{\bar{x}}$) against 164 3±1 0 cm and in weight averaging 75 2±1 7 kg against 64 0±1 8 kg while they were about alike in average age—42 7±2 5 years against 43 0±2 7 The

values for these characteristics were spread over about the same ranges in both series

Cubing the height did not strengthen any of the correlations in the present study and the same was true for the series of 50 year old men The body surface area was not better correlated than the height and/or weight with the lung mechanics

Lung volumes

Total lung capacity *TLC* was positively related to height both in the men and women the determination coefficient (R^2) amounting to 0 29 and 0 38 respectively

Residual volume *RV* was positively related both to the *TLC* and age in both sexes the determination coefficient amounting to 0 69 and 0 41 respectively It was not significantly correlated with either height or weight either alone or combined with age notwithstanding that the *TLC* depended on height

Functional residual capacity *FRC* was positively correlated with the height in the men and positively with the height and negatively with the weight in women R^2 amounting to 0 24 and 0 31 respectively

Vital capacity *VC* was positively correlated with height and negatively correlated with age It was also correlated with the *TLC* the R^2 for the *TLC* amounting to 0 84 for the men and to 0 81 for the women and for height to 0 49 for both The regression with height gave an *RSD* of 0 59 and 0 51 for men and women respectively

Forced expiratory volume FEV_1 was positively correlated with height and negatively with age, R^2 amounting to 0 52 and 0 48 respectively It was not correlated with weight It was strongly positively correlated with *VC*, and in this regression in the men also negatively correlated with age

$FEV_1\%$ was negatively correlated with age and height (R^2=0 29) in the men This could be seen already in the equation for FEV_1, where the constant for height was much lower than in the equation for *VC* The women also showed a tendency to a negative correlation with age, but it was not statistically significant

Maximum expiratory flows

The peak expiratory flow in the men was negatively correlated with age and positively with weight (R^2=0 40) but it was not significantly related to other anthropometric variables, such as height or to *TLC* or *VC* In the women it was significantly correlated with both *TLC* and *VC* but not with age

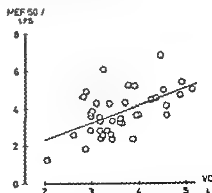


Fig 2 Relationship between maximum expiratory flow at 50 per cent of vital capacity ($MEF50\%$) and vital capacity (*VC*) in control women The regression equation in table 3 (no 28)

The $MEF50\%$ was also significantly related to TLC and VC in the women, VC being the best independent variable ($R^2=0.30$). In the women it was also weakly negatively correlated with age alone ($R^2=0.11$). Its only significant connection in the men was a negative one with age ($R^2=0.28$).

$MEF25\%$ was only significantly (negatively) correlated with age in the men but in the women it was also correlated (positively) with TLC and VC, VC giving the highest determination coefficient ($R^2= =0.26$).

The division by $1/4\ VC$ used to get a value for the slope of the flow volume curve during the two last quarters of expiration should help to eliminate the effect of the lung volumes. When this was done only a negative relationship with age persisted for $4\ MEF25\%/VC$ while $4(MEF50\%-MEF25\%)/VC$ was not significantly connected with age or anthropometric variables. The quotient obtained

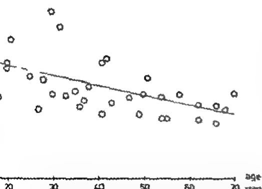


Fig. 3. Relationship between maximum expiratory flow at 50 per cent of vital capacity ($MEF50\%$) and age in control men. The regression equation in table 3 (no. 23).

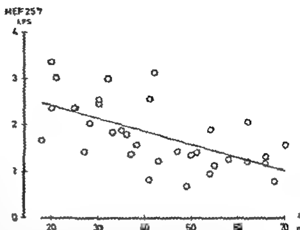


Fig. 4. Relationship between maximum expiratory flow at 25 per cent of vital capacity ($MEF25\%$) and age in control men. The regression equation in table 3 (no. 29).

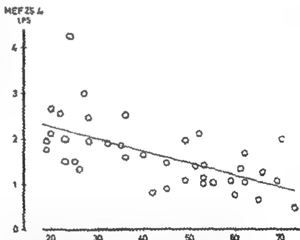


Fig. 5. Relationship between maximum expiratory flow at 25 per cent of vital capacity ($MEF25\%$) and age in control women. The regression equation in table 3 (no. 30).

from the two expressions should, as mentioned on page 24, provide a measure of the curvilinearity in the flow volume curve. It is evident from the relationships

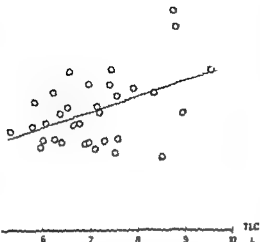


Fig 6 Relationship between dynamic compliance (Cdyn(l)) and total lung capacity (TLC) in control men The regression equation in table 3 (no 41)

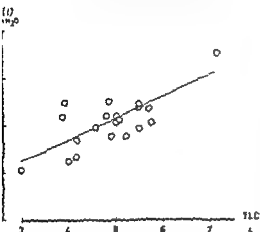


Fig 7 Relationship between static compliance (Cst(l)) and total lung capacity (TLC) in control women The regression equation in table 5

shown in equations 39 and 40 that with advancing age the last fourth of the curve descends more gradually than in the foregoing quarter

Dynamic and static compliance

Dynamic compliance Cdyn(l) was positively correlated with *TLC* alone and with *VC* combined with age, this combination giving the best values for the determination coefficients (R^2=0 36 and 0 19 in the men and women respectively) It was also positively correlated with *FRC* in the women (R^2=0 11)

Static compliance Table 4 and fig 22 on page 65 show the average relationship in 20 control women between *Pst(l)* and lung volume Testing the relationship between *Pst(l)* and age at 30 40, 50, 60, 70, 80, 90 and 100 per cent of *TLC* and at 10 to 100 per cent of *VC* with linear regression analysis revealed only a significant correlation at 100 per cent of *TLC* (or *VC*)

The regression equation was

$$Pst(l)100\%TLC = -0\ 21 \underset{(\pm 0\ 06)}{\ } age + 37\ 8$$

$$R^2 = 0\ 37,\ RSD = 4\ 52$$

There was a tendency to age dependence at 90 per cent of *VC* but not at any other percentage of *TLC* or *VC* in this series judging from both graphic and statistical analysis

The static compliance—*Cst(l)*— calculated with the *FRC* and tidal volume recorded during measurement of *Cdyn(l)* varied between 0 105 and 0 351 *L*/cm H_2O It was related to height (R^2=0 21) *TLC* (R^2=0 53) *FRC* (R^2=0 31) and age in combination with *VC* (R^2=0 52) and also to *VC* alone (table 5) Fig 7 depicts the relationship between *Cst(l)* and *TLC*

The following shows the regression of $Cst(l)$ on $Cdyn(l)$ in these 20 women

$$Cst(l) = +0.8423 \quad Cdyn(l) + 0.0592,$$
$$(\pm 0.1500)$$
$$R^2 = 0.64, \; RSD = 0.0329$$

See also fig 23 on page 65

Conductance

Inspiratory conductance The inspiratory conductance instead of resistance, was chosen for the regression analysis, as previous studies have shown this to be directly or almost directly proportional to certain lung volumes —*FRC* for example In the men $Gi(l)$ was only connected with *VC* ($R^2=0.40$) while in the women it was weakly correlated with both *TLC*, *FRC* and *VC* R amounting to 0 15 0 11 and 0 11 respectively

Analysis was also made of the connection between anthropometric variables and the function $Ri(l)$ $Cdyn(l)$, which may be assumed to represent the sum of the different time constants of the lungs $Ri(l)$ $Cdyn(l)$ rose slightly with increasing age in the men ($R^2=0.13$) but not in the women

Peripheral airway conductance

For calculating *Gus* correction was first made for the effect of alveolar gas compression on the *Vmax* values on the flow volume curves of the 20 control women The results are seen in table 6 and fig 24 on page 65

Gus was then calculated as *Vmax* corrected for alveolar gas compression divided by $Pst(l)$ for every 10 per cent of *VC* up to 70 per cent of *VC* The results are given in table 7 and fig 8

The connection between *Gus* on the one hand and age *TLC* and *VC* on the other was tested by bivariate and trivariate

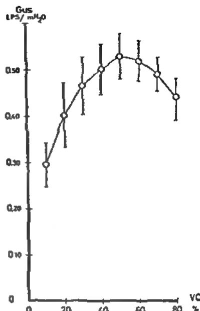


Fig 8 Average relationship between peripheral airway or upstream conductance (*Gus*) and vital capacity (*VC*) in control women

regression analysis The statistically significant ($p<0.05$) correlations found are given in table 8 *Gus* was only dependent on age at 10 20 and 40 per cent of *VC* ($R^2=0.26$ 0 22 and 0 29 respectively) and on age, *TLC* and *VC* separately at 50 and 60 per cent of *VC* and on *TLC* and *VC* separately at 70 per cent of *VC* *VC* gave the highest values for R^2—0 32 0 37 and 0 39 for 50 60 and 70 per cent of *VC*, respectively No significant correlations emerged with age together with *TLC* or *VC* as independent variables *TLC* and *VC* were each negatively correlated with age R^2 being 0 20 and 0 55 respectively

On multiplying the coefficient for age in the regression equations with the value for the range in age in the series (48 years) and the coefficient for *VC* with the range in

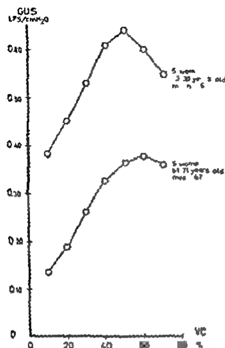


Fig. 9. Average relationship between peripheral airway or upstream conductance (Gus) and vital capacity (VC) in the 5 youngest and 5 oldest women in the control series.

VC (=3.52 L). VC acquired a little stronger influence than age on Gus at 50 per cent of VC (0.41 against 0.35 LPS/cm H_2O) and at 60 per cent of VC a still stronger effect (0.46 against 0.28 LPS/cm H_2O).

The peripheral airway conductance at different percentages of VC for the 5 youngest and 5 oldest subjects of this series is plotted in fig. 9.

Discussion

Lung volumes

In 1963 Grimby & Soderholm found that in men TLC was correlated with height ($b=+0.0701$ at $BTPS$ as are all the figures) and weight ($b=-0.019$) but in the present study it was only related to height ($b=+0.0824$); in women they found that it was related to age ($b=-0.017$) and height ($b=+0.0738$), but in the present study it was only related to height ($b=+0.0774$). The predicted means for the present control series amount to 7.33 L for the men when inserted into the equations of these authors, and to 5.04 L for the women. These values do not differ significantly from the values found here (7.10 L and 5.06 L, respectively).

In the foregoing authors' regression equations for RV, age has positive coefficients both for men and women, but the coefficients are larger for the men than for the women ($b=+0.024$ v. $+0.008$, $BTPS$), a tendency corresponding with the results in the present study ($b=+0.021$ v. $+0.013$). The predicted mean values for the present control series according to Grimby & Soderholm are 1.95 L for the men and 1.41 L for the women; these do not differ significantly from the values found (1.60 L v. 1.48 L).

In Grimby & Soderholm's series FRC was positively correlated with height and negatively with weight both in the men and women, and in the men it was also positively correlated with age. The coefficients for height corresponded, while the ones for weight were lower in the present study and as mentioned the coefficient was not significant for the men. The predicted mean values for FRC calculated according to Grimby & Soderholm are 3.07 L for the present men and 2.34 L ($BTPS$) for the women. The second of these values differs significantly from the one found in this study—2.64 L ($p<0.01$). This may have been because the ERV was measured in different ways—with the Servo

Spirometer in the present study and with the helium method in Grimby & Söderholm's study

Several authors have confirmed Hutchinson's observation from 1846 that VC is better correlated with height than with any other anthropometric measure. Except for series containing young subjects, a linear function of height fits better than the height cubed. This is probably because adults vary less in height than do children. The following shows the regression equations various authors including myself have found for VC.

In the series of 50 year old men regression analysis (table 4) showed that height by itself was the best anthropometric independent variable for VC (R^2=0 64, RSD=0 588) and the coefficient corresponded fairly well with that in the smaller series in the present study and with those found by other authors. In all the studies the RSD in the regression equations amounted to about 0 5 L.

On the next page the values for some of the ventilatory variables observed in the series of 50 year old men are compared with the predicted values judging by the

		RSD
Men		
Berglund *et al* (1963)	VC =0 0[illegible]3 H − 0 022 A − 3 09	0 5[illegible]
Ferris *et al* (1965)	FVC = 0 031 H − 0 02[illegible] A − 3 5[illegible]	0 [illegible]0
Goldman & Becklake (1959)	VC = 0 064 H − 0 031 A − 5 34	0 49
Kory *et al* (1961)	VC = 0 052 H − 0 0[illegible]2 A − 3 60	0 58
Needham *et al* (1954)	VC = 0 048 H − 0 039 A − 2 12	0 44
Pemberton & Flanagan (1956)	FVC = 0 048 H − 0 029 A − 2 42	—
Present series	VC = 0 067 H − 0 017 A − 5 8	0 59
Women		
Berglund *et al* (1963)	VC = 0 044 H − 0 0[illegible]4 A − 2 59	0 44
Ferris *et al* (1965)	FVC = 0 033 H − 0 0[illegible]3 A − 1 40	0 46
Goldman & Becklake (1959)	VC = 0 052 H − 0 018 A − 4 [illegible]	0 43
Needham *et al* (1954)	VC = 0 044 H − 0 0[illegible]2 A − 3 00	0 36
Present series	VC = 0 050 H − 0 01 A − 3 83	0 [illegible]1

The different authors have used different apparatuses, some have measured the forced vital capacity (FVC) and some the slow VC, and in addition their subjects have varied in body structure, the way they were selected and other features. All the same their results correspond well, particularly for women. The male and female controls of the present study, however, showed a tendency to less dependence on age than the series of other authors.

regression equations obtained in the present series and that of Berglund, Birath, Bjure, Grimby, Kjellmer, Sandquist and Soderholm (1963).

As seen, the observed and predicted values for the different ventilatory variables differed very little.

All three series contained both smokers and nonsmokers. It has been found that both VC and FEV_1 and other variables have different regression lines depending

	Observed values in 50 year old men		Predicted values according to Present investigation		Berglund *et al*
	$\bar{x}$	s_x	Eq No	x	x
Height cm	1[illegible]6	6			
Weight kg	74 1	9 2			
VC L	4.90	0 69	9	5 01	5 01
FEV_1 *L*	3 [illegible]6	0 54	13	3 [illegible]	3 6[illegible]
FEV_1%	[illegible]6 9	6 9	17	76 [illegible]	78 1
*MEF*50% *LPS*	4 60	1 19	23	4 64	
*MEF*25% *LPS*	1 68	0 54	29	1 [illegible]9	
4(*MEF*50% − *MEF*25%)/*VC* 1/sec	2 39	0 [illegible]6		2 49	
4 *MEF*25%/*VC* 1/sec	1 38	0 44	37	1 26	
MEF 50% − *MEF*25%/*MEF*25%	1 86	0 [illegible]2	49	2 14	

on smoking habits (Ferris Anderson & Zickmantel 1965) These variables differ according to smoking habits even among subjects without respiratory symptoms (Wilhelmsen & Tibblin 1966) In the study by Ferris *et al* (1965), the regression lines for the different groups cut each other at or shortly after the age of 40 The groups in the present study are too small for judging the effect of smoking on the respiratory mechanics Thus the controls included only one man who smoked 15–24 g of tobacco a day As the heart series contained both smokers and nonsmokers it was arranged for the control series to contain both As seen from page 14, the heart patients consumed slightly less tobacco on the average than the control subjects

The following shows the regression equations for FEV_1 found in the present and other series

		RSD
Men		
Berglund *et al* (1963)	FEV_1 = 0 028 *H* − 0 026 *A* − 1 10	0 53
Cotes *et al* (1966)	FEV_1 = 0 033 *H* − 0 033 *A* − 1 12	0 45
Ferris *et al* (1965)	FEV_1 = 0 036 *H* − 0 0[illegible] *A* − 1 65	0 49
Kory *et al* (1961)	FEV_1 = 0 03 *H* − 0 028 *A* − 1 [illegible]9	0 52
Ringqvist (1966)	FEV_1 = 0 035 *H* − 0 028 *A* − 0 3[illegible]	0 48
First four series combined according to Cotes *et al* (1966)	FEV_1 = 0 036 *H* − 0 031 *A* − 1 41	
Present series	FEV_1 = 0 038 *H* − 0 0[illegible]2 *A* − 1 81	0 46
Women		
Berglund *et al* (1963)	FEV_1 = 0 0[illegible]9 *H* − 0 030 *A* − 0 [illegible]9	0 40
Ferris *et al* (1965)	FEV_1 = 0 025 *H* − 0 0[illegible]7 *A* − 0 62	0 40
Ringqvist (1966)	FEV_1 = 0 036 *H* − 0 028 *A* − 1 [illegible]1	0 38
Present series	FEV_1 = 0 028 *H* − 0 027 *A* − 2 [illegible]2	0 48

As in the case of the VC the different series had remarkably similar regression coefficients. Thus the coefficient for height in the 50 year old men was 0.040 and in the present control series 0.038. The coefficient for age was lower in the present series than in the others. This may be because the others used other types of subjects than the controls chosen for the present study. Subjects with dyspnea and cough were excluded from the control series and dyspnea, in particular, is more common among the old than the young.

The 50 year old men showed a significant connection between FEV_1 and height and height^3 and VC but R^2 was only about 0.50 for the first two and 0.58 for VC.

The correlation between FEV_1 and age being weaker in the present series than in the other series, $FEV_1\%$ was also less dependent on age in the present series. $FEV_1\%$ is negatively correlated with VC according to Ringqvist (1966) if the coefficients in the regression are calculated from the logarithms for the variables. This was borne out by the 50 year old men who also showed a weakly negative correlation between $FEV_1\%$ and VC. Thus in the case of large vital capacities division with VC leads to overcompensation of the volume factor. This probably explains the high values for $FEV_1\%$ in women reported by Gaensler (1951), Leuallen & Fowler (1955), Miller *et al* (1959), Grimby & Soderholm (1963) and Ringqvist (1966).

Maximum expiratory flows

PEF was positively correlated with weight in the present study, but not with the height in men. Kory *et al* (1961) did not find any correlation between PEF and anthropometric variables in a study of 369 men and consequently gave only one lower normal limit for PEF for men between the ages of 20 and 65—3.3 LPS. In the present study 4.68 LPS was the lowest value found in the men. The series of 50 year old men showed a weakly positive correlation between PEF and VC ($R^2=0.13$). PEF in these men was measured with the meter devised by Wright & McKerrow (1959). Several series, as well as the men in the present study, have shown a negative correlation between the PEF and age in adults (Higgins 1957, Lockhart Smith, Mair & Wilson 1960, Selby & Read 1961, Tinker 1961) but Goldsmith (1958) did not succeed in finding any correlation between the two. Ferris *et al* (1965) found that PEF was negatively correlated with age and positively with height in both men and women.

$MEF50\%$ was negatively correlated with age in the present series. Leuallen & Fowler (1955) and Birath *et al* (1963) found the same for the $MEF25\%$–75%, and Kory *et al* (1961) observed that $MEF25$–75% was weakly positively correlated with height and negatively with age. Leuallen & Fowler (1955) and Kory *et al* (1961) found significant correlations between $MEF25$–75% and VC which corresponds to the findings in the present series for women in whom $MEF50\%$ was positively correlated with TLC and with VC. In the 50 year-old men the $MEF50\%$ was weakly positively correlated with VC ($R^2=0.09$). The correlation between $MEF25$–75% as well as $MEF50\%$ and lung volumes probably stems from the connection between airway conductance and lung volumes.

No multiple regression studies have been made of the $MEF25\%$ with consideration given to age, anthropometric variables and lung volumes. The series published by Sobol & Emirgil (1964) showed a distinct negative correlation with age in agreement with the observations in the present study. The present study showed that $MEF25\%$ depended on TLC and VC, but not to such a large extent as $MEF50\%$, and that it was much more dependent on age than on lung volumes both in men and women. The $MEF25\%$ values for the 50 year-old men were not correlated with anthropometric variables or VC.

The slope of the flow volume curve as expressed by $4(MEF50\%-MEF25\%)/VC$ and $4\ MEF25\%/VC$, showed no correlation with anthropometric variables or VC in the series of 50 year old men in which the age factor was eliminated. These indices should therefore be suitable for judging the slope of the curve since they eliminate the effect of lung volumes.

In all the comparable regression equations involving age discussed up to now (except nos 38 and 48) the coefficients for age were less negative in the women than in the men.

Dynamic and static compliance

The following figures have been found for $Cdyn(l)$ on slow respiration in normal subjects: 0.200–0.670 (Buytendijk 1949), 0.140–0.300 (Mead & Whittenberger 1953), 0.090–0.330 (Marshall 1957), 0.100–0.390 (Ehrner 1960), 0.097–0.301 $L/cm\ H_2O$ (Petit, Senterre, Boccar, Delrez & Damoiseau 1962) and 0.098–0.402 in the present study.

In normal persons the compliance depends on the size of the lungs, probably not only on their gas volume but also on the mass of their tissue (Frank *et al.*, 1956). The lung volumes utilizable for calculating this relation depend, in turn, on the compliance of the lungs and also on mechanical factors in the chest wall. Marshall (1957) found that $Cdyn(l)$ was positively correlated with height, body surface area, FRC, RV, inspiratory reserve volume and VC in a series of 50 normal subjects—41 males and 9 females—selected so as to get a wide range in lung volumes and aged between 9 and 12 and 19 and 35 years respectively.

Cook, Helliesen & Agathon (1958) collected data from newborn infants, children between 5 and 17, and young adults, and found that $Cdyn(l)$ was positively correlated with FRC, VC and the predicted lung weight, except in the case of the newborn. Ehrner & Nisell (1959) observed that $Cdyn(l)$ was positively correlated with height, VC, FRC, TLC and maximum breathing capacity in 16 men and 15 women between 16 and 79 with no respiratory symptoms. Brown *et al.* (1954) found that the determination coefficient for the correlation between the elasticity of the lung and $1/VC$ was 0.45 in 16 normal men and women. Ringqvist (1966) determining the dynamic compliance in 82 normal men and 76 normal women between 18 and 83 found determination coefficients of 0.25 and 0.20 for the correlation between $Cdyn(l)$ and TLC, and of 0.24 and 0.14 between $Cdyn(l)$ and VC.

The average values and ranges for $Cdyn(l)$ were the same in the present series as in others, but the correlations with different lung volumes were all weak, mostly like those discovered by Ringqvist (1966). It is noteworthy that $Cdyn(l)$ was

significantly correlated with *FRC* only in the women, and that this variable only explained 11 per cent (R^2=0 11) of the variance between the subjects In the present series *Cdyn(l)* was most closely correlated with age and *VC* The age coefficient was three times larger for the men

In agreement with the findings in the present study Cohn & Donoso (1963) found that *Cdyn(l)* rose with age The reason their findings disagreed with those of Frank *et al* (1957) is probably that 72 per cent of the latters subjects were women As mentioned age seems to have less effect on *Cdyn(l)* in women than in men Frank *et al* (1957) studying pressure volume curves over the whole *VC* in men and women noted a shift to the left in the compliance curve with increasing age—that is the *Pst(l)* was less negative for a given absolute lung volume between the ages of 50 and 89 than between 22 and 47 Pierce and Ebert (1958) also noted that the *Pst(l)* was less negative in older than younger men at a given lung volume Permutt and Martin (1960) comparing *Pst(l)* with absolute lung volumes in men between 21 and 76 years observed no change in the slope or position of the pressure volume curve with advancing age but observed that the older men changed transpulmonary pressure between *RV* and *TLC* as much as the young men Mead *et al* (1967) studying *Pst(l)* in relation to percentage of *TLC* in four groups of men between 13 and 61 years old found that *Pst(l)* at a given lung volume decreased markedly from the youngest to the next youngest group but without an associated change in *RV* The next older group exhibited further reduction in *Pst(l)* but an increase in *RV* and the oldest group showed no further reduction in *Pst(l)* but an increase in *RV* The results were not subjected to variance analysis

In comparing the figures different authors have given for *Pst(l)*, one must take into account the different ways they have calculated their values Mead *et al* (1967) like myself measured *Pst(l)* at certain percentages of *TLC*, and the other authors at absolute lung volumes which change with advancing age Furthermore, none of the studies have been made on large series Both the present study and that of Frank *et al* and Permutt & Martin however showed a significant decrease in *Pst(l)* at 100 per cent of *TLC* with age On the other hand it is not certain that the esophageal pressure method gives reliable results at *TLC*

Different authors have found the following values for *Cst(l)* at *FRC* 0 160–0 310 L/cm H_2O (Stead Fry & Ebert 1952) 0 09–0 33 (Frank *et al* 1956) and 0 105–0 451 (present study)

Frank *et al* (1956) found that *Cst(l)* was correlated with height and *VC* with an R^2 of 0 49 in 38 healthy men and 32 healthy women aged 18–39 which agrees with the present results The present finding that *Cst(l)* was positively correlated with age and vital capacity agrees with the finding for *Cdyn(l)* Mittman, Edelman Norris & Shock (1965) did not find the dependence between age and compliance observed by Pierce & Ebert (1958) and suggested that this might have been because Pierce & Ebert s series contained subjects with chronic obstructive pulmonary disease But the present series contained no subjects of this kind yet it too showed a positive correlation between these variables It is true that the pressure

volume curve for the whole *VC* revealed no age dependence in the middle part. Yet the *Cst(l)* at *FRC* did so, probably because the *FRC* increase with age, which in turn would mean that *Cst(l)* is measured on differently inclining parts of the sigmoid shaped pressure volume curve.

Davidson, Wasserman, Lillington & Schmidt (1966) observed, however, that the static compliance increased with increasing age in rabbits. The anatomic background of these changes with age is not yet clear, and different studies on the elastic fibers have given different results (Wright, 1961; Pierce & Hocott, 1960; Kohn, 1964).

Conductance

Studies of small series for the normal values for airway resistance, as measured with a body plethysmograph and panting, have given figures between 0.91 and 1.60 cm H_2O/LPS in men and between 0.99 and 1.73 in women (DuBois, Bothelho & Comroe, 1956; Marshall & DuBois, 1956; Briscoe & DuBois, 1958; Schmidth & Cohn, 1961; Nadel & Comroe, 1962; Jaeger & Otis, 1964).

The total pulmonary resistance in normal subjects, as measured via the esophageal pressure, has been given as 1.2–2.8 (Mead & Whittenberger, 1953), 1.2–3.4 (Frank *et al.* 1957), 1.2–4.8 (Marshall 1957) and 0.8–3.7 (Ebner 1960). These ranges for subjects with healthy lungs thus agree fairly well with the range in the present controls (table 2), viz. 0.55–3.33 cm H_2O/LPS.

Mead & Whittenberger (1953), studying three normal subjects, noted that the resistance fell with increasing lung volumes within the range of *VC*. Fry *et al.* (1954) confirmed this observation. Marshall (1957) stated that *R(l)* was not correlated with the *FRC* in adults with an *FRC* above 2.5 *L*, but that it rose with an *FRC* below 2.5 *L*. Helliesen *et al.* (1958) reported that the airway resistance was negatively correlated with height in 77 normal children between 5 and 17. Briscoe & DuBois (1958), studying 26 normal subjects between 4 and 87 with a body plethysmograph, noted that the airway conductance was *approximately directly proportional* to the amount the lung was inflated (*FRC*). As they found this to be true of all ages, they concluded that the conductance was not related to age, but to *FRC*. Cheng, Godfrey & Shepard (1959), using the air flow interruption method, observed that the resistance stayed relatively constant until about 80 per cent of the *VC* had been expired, and then rose abruptly to extremely high values. A recent study by Brunes & Holmgren (1966) with a body plethysmograph showed a close connection between airway conductance and lung volume in young women, the same as noted by Briscoe & DuBois (1958).

The results of the present study correspond relatively well to those from other studies of total pulmonary resistance and conductance.

Because the present control series agreed fairly well with the others, particularly the 50 year old men, in means and regression equations for lung volumes and different variables of maximum expiratory flow, such as *PEF*, *MEF*50% and *MEF*25%, it should be justifiable to consider the compliance and conductance values obtained from them as control values, suitable for comparing with the results in different diseases.

Peripheral airway conductance

Mead *et al* (1967) corrected their *Gus* values for lung volume by dividing the flows by *TLC*. As the *Gus* was only significantly correlated with *TLC* and *VC* at 50 to 70 per cent of *VC* in the present study, it was not justifiable to make this correction for all the percentages of *VC*. At 50, 60 and 70 per cent of *VC* *Gus* could be predicted from the regression equations with *VC* (table 8). As seen from table 8, the constants are small signifying that the *Gus* was nil near zero *VC*.

Mead *et al* (1967), making an extensive theoretical study of the flow-volume-pressure relationship in healthy subjects found that *Gus* decreased nearly linearly with lung volume in older subjects appearing to extrapolate to zero near *RV*, a finding verified in the present study (fig 9). This suggests that the upstream segment collapses altogether at *RV* in old people.

RESULTS IN PATIENTS WITH RHEUMATIC VALVULAR DISEASE

Respiratory symptoms

As seen from table 1, 3 men and 5 women in the main series of patients (c) had cough and sputum 3 months a year (For simplicity's sake these symptoms are labelled 'chronic bronchitis' in the following even though they were combined with heart disease which means departing from the current definition) These were 46 years old, on the average According to the criteria of the New York Heart Association, they showed no greater functional disability than the whole series of patients with rheumatic valvular disease Respiratory symptoms were more common, however in the cases of high pressure in the pulmonary artery ($\bar{P}_{PA}$) and left atrium ($\bar{P}_{LA}$) and of elevated pulmonary vascular resistance (PVR) Thus more of these patients than the others had cough and sputum for 3 months a year, still more had cough and sputum for shorter periods of the year, and even more complained of wheezing, particularly on exertion The breakdown of the 51 patients is seen below

Three patients out of 14 in the other two series had cough and sputum 3 months a year Thus there were 11 cases (17%) with these symptoms among all 65 Seven of the 11 patients smoked, but only moderate amounts while only 8 of the other 54 patients were smokers

Comments It has long been considered that cough with or without sputum and wheezing is more common than normal in mitral valvular disease But as yet no comparison has been made between the frequency of these symptoms in mitral valvular disease and a random sample of the population

A recent epidemiologic study of 50 year old men in Goteborg (Tibblin & Wilhelm

		$\bar{P}_{PA}$ mm Hg			$\bar{P}_{LA}$ mm Hg			PVR mm Hg/(L/min)		
		≤19	20–29	≥30	≤9	10–19	≥20	≤1.4	1.5–2.4	≥2.5
Cough and sputum	No	3	1	4	1	3	4	2	3	3
3 months a year	%	16	6	29	9	14	22	10	16	25
Cough and sputum	No	[illegible]	4	4	1	4	5	3	3	4
less than 3 months a year	%	11	22	29	9	18	28	15	16	33
Wheezing most days	No	5	7	11	2	7	14	5	9	[illegible]
or only sometimes	%	28	39	79	18	32	78	25	47	75
Total	No	19	18	14	11	22	18	20	19	12

sen, 1967) has shown that 8 per cent of them had cough and sputum 3 months a year (chronic bronchitis). Chronic bronchitis has been reported to be more common among men than women (Oswald & Medvei, 1953; Higgins, 1957; Higgins & Cochran, 1958; Fletcher, Elmes, Fairbairn & Wood, 1959; Ferris & Anderson, 1962) but a recent study (Julin & Wilhelmsen, 1967) has shown the same frequency in both sexes. The differences between the sex ratios found may be due to differences in geography. Eleven of the present patients had chronic bronchitis, which is a significantly high number, judging by the results of the aforementioned study of 50 year old men ($\chi^2=6.92$, $p<0.01$).

The heart cases were not selected in a way that could have caused a misleadingly high frequency. Some of the subjects came from other places than Göteborg, but judging by the incidence of premature pensions in Sweden as a whole (Wilhelmsen, 1967) they should have had bronchitis less often than the inhabitants of Göteborg.

The rise in the frequency of respiratory symptoms with a rise in the $\bar{P}_{PA}$, $\bar{P}_{LA}$ and PVR points to bronchitis increasing in frequency with advancing heart disease.

Aber (1963) noted that the rate of recurrent bronchitis for at least three years increased with increasing functional disability in 84 patients with mitral stenosis, finding rates of 0, 39.5, 51.4 and 66.6 per cent in functional classes I to IV. Only 5 of his patients with bronchitis had significantly lowered values for $FEV_1\%$ and maximum voluntary ventilation and he concluded from this that the rise in the frequency of bronchitis from class to class was not the reason for the increasing functional disability from class to class.

As pointed out on page 14, all the cases with cough and sputum 3 months a year were excluded from the main series when values for pulmonary function were related to functional disability, changes in pulmonary roentgenograms and pulmonary hemodynamics to eliminate any effect these symptoms may have had on these values.

Procedure for comparing variables

For analyzing the effect of different factors on the lung mechanics in heart disease the main patient series was divided into ones with and without symptoms of chronic bronchitis. Then the patients with *no* symptoms of chronic bronchitis were subdivided (a) into different functional classes, (b) according to the observations in pulmonary roentgenograms and (c) according to the size of the heart and to different hemodynamic variables. The significance of the pulmonary blood volume was also studied.

It was possible to use multiple regression analysis for studying the significance of the hemodynamic variables and the size of the heart, as these are continuous variables. The pulmonary blood volume was not measured in the whole series, so this variable was not included in the multiple regression analysis. The absolute values for the different variables were used for most of the statistical analysis. In some calculations the observed value divided by the predicted normal value in per cent was also used so as to

be able to combine the results for the men and women

To calculate the predicted values from the results in the controls the regression equations containing age and anthropometric variables and lung volumes were used and the equations giving the highest values for the determination coefficients were used—that is the regression equations numbers 1, 2 3, 4, 5 6 9, 10 13 14 17 18 19, 22 23 28 29, 30 43 44, 48 and 51 in table 3 The predicted value for *RV*, *Cdyn(l)* and *Gi(l)* in both sexes and *PEF* and *MEF50%* in the women, which were correlated with different lung volumes, were calculated both from the predicted and observed value for the lung volume in question

Lung mechanics in relation to symptoms

Chronic bronchitis

The means for different variables of lung mechanics in the patients divided into those with and those without cough and sputum 3 months a year (chronic bronchitis) were compared with the predicted normal values according to the regression equations and the differences tested according to the formula on page 30 The results for the men are shown in table 10, and for the women in table 11

The means of the observed in per cent of predicted values for some of the variables for the men and women combined are shown at the bottom of this page

Only a few of the patients had cough and sputum for three months a year 3 men and 5 women Nevertheless some of their mean values differed significantly from the predicted normal values *RV* was higher than normal for both sexes in this group if the predicted value was based on the observed value for *TLC* In addition most of the mean values obtained on tests for bronchial obstruction in the 5 women differed from the normal values *MEF25%* varied between 22 and 99 per cent of the predicted normal value

	Series without cough and sputum 3 months a year (n=43)			Series with cough and sputum 3 months a year (n=8)		
	$\bar{x}$	$\sigma_{\bar{x}}$	No of cases with values ≥100% of pred	$\bar{x}$	$\sigma_{\bar{x}}$	No of cases with values ≥100% of pred
TLC	88 1	2 0	6	90 8	5 1	1
VC	80 9	2 3	6	73 0	4 8	[illegible]
MEF25%	72 2	5 7	7	55 3	10 1	0
Cdyn(l)	73 8	3 8	6	77 1	7 8	1
Cdyn(l)	90 1	4 4	17	92 9	7 3	3
Ci(l)	63 7	3 8	4	57 9	12 5	2
Gi(l)	74 4	4 2	8	64 8	12 3	3
Sign test $p<0.05$ if			(14/29)[b]			(0/5)[b]

[a] Predicted values based on predicted values for *TLC* (women) or *VC* (men)

[b] These figures give the porportions the sign test requires for significance at the 5 per cent level

In the series without cough and sputum for three months a year the values were alike for both sexes *TLC* was lower than would be expected from the height *RV* was lower than normal in the women judging by the predicted *RV* based on the predicted value for *TLC* When the predicted value was based on the observed *TLC* *RV* was higher than normal in both the men and the women resulting in an above normal *RV/TLC* index of 0 31 and 0 35 for the men and women respectively as opposed to 0 26 and 0 29 in the control subjects *VC* was lower than would be expected from the age and height and likewise FEV_1 while $FEV_1\%$ lay within normal limits

PEF and *MEF*50% were lower than normal in the men but *MEF*25% was not significantly low in their case as it was for the women *PEF* and *MEF*50% were low in the female heart patients compared with the predicted values based on the predicted *VC* but not low compared with the predicted values based on the observed *VC*

Cdyn(l) was lower in both the men and women than the predicted values based on the predicted *VC*, but only in the women when the observed *VC* was used for the calculation

Gi(l) was low in both the men and women compared with the predicted values based either on the predicted or observed lung volumes

Fig 10 and fig 11 show the maximum expiratory flow volume curves for the men and women without chronic bronchitis in relation to the predicted normal curves The normal values for the maximum flow variables were reckoned from the predicted normal lung volumes with consideration given to age height and weight

The patients with chronic bronchitis did not differ significantly from the ones without bronchitis in any variable of lung mechanics judging from the relationship between predicted and observed values

Functional disability

As only 2 patients belonged to the New York Heart Association functional class IV functional classes III and IV were

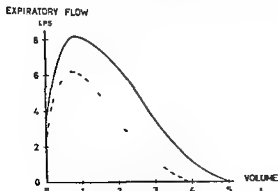


Fig 10 Maximum expiratory flow volume curve for men with rheumatic valvular disease (broken line) compared to the predicted normal curve (solid line)

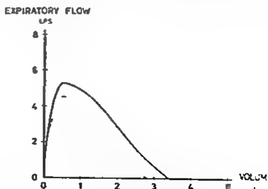


Fig 11 Maximum expiratory flow volume curve for women with rheumatic valvular disease (broken line) compared to the predicted normal curve (solid line)

	Functional class I ($n=9$)			Functional class II ($n=22$)			Functional classes III+IV ($n=12$)			Variance analysis
	$\bar{x}$	$s_{\bar{x}}$	No. with >100% of pred.	$\bar{x}$	$s_{\bar{x}}$	No. with ≥100% of pred.	$\bar{x}$	$s_{\bar{x}}$	No. with ≥100% of pred.	$p<$
TLC	93.5	3.9	2	87.7	2.3	4	77.4	3.9	1	0.01
VC	92.1	4.9	3	83.6	2.4		67.4	3.1	0	0.01
*MEF*50%	99.6	11.6	5	73.5	7.5	3	49.1	8.1	1	0.01
Cdyn(l)[a]	84.5	[illegible]	[illegible]	79.0	4.9	4	56.7	6.7	0	0.01
Cdyn(l)	96.5	6.9	6	94.7	6.6	8	76.6	6.1	3	—
Gt(l)[a]	68.8	8.6	1	64.6	5.5	1	58.7	7.0	1	—
Gt(l)	77.0	9.7	–	73.5	6.1	3	74.3	8.1	[illegible]	—
Sign test $p<0.05$ I			(1/8)[b]			(5/17)[b]			(?/10)[b]	

[a] Predicted values based on predicted *TLC* or *VC*

[b] These figures give the proportions the sign test requires for significance at the 5 per cent level.

pooled. The means for P_{pA} in the resulting three classes were 11.3, 12.7 and 16.5 mm Hg for the men and 13.0, 15.6 and 22.6 mm Hg for the women. The corresponding values for *PVR* were 1.73, 1.81 and 3.38 and 0.90, 1.05 and 6.00 mm Hg/(*L*/min) in the respective classes.

The values for the 3 class I women did not differ from the predicted normal values, but the 6 class I men had a lower *TLC*, *VC*, *PEF*, *MEF*50% and *Gt(l)* than predicted (tables 12 and 13). *Cdyn(l)* was lower than predicted calculating from the predicted *VC*.

In functional class II both the men and the women had subnormal values for *TLC*, *VC*, *FEV*$_1$, *MEF*50%, *Cdyn(l)* and *Gt(l)* when the predicted values for the last two were based on the predicted *VC* or *TLC*. Testing the observed in per cent of the predicted values for the pooled sexes with the sign test brought out deviations from normal in *TLC*, *VC*, *MEF*25% and also in *Cdyn(l)* when the observed value was compared with the predicted *Cdyn(l)* based on the predicted *VC* and in *Gt(l)* whatever way the predicted value was reckoned. The figures are given above.

Functional class III+IV contained 8 women but only 4 men. Despite the small number of men both sexes deviated from the normal in *TLC*, *FRC*, *VC*, *FEV*$_1$, *PEF* and *MEF*25%. The *MEF*50% was only subnormal in the women, and only when the predicted value was based on the predicted *VC*. The deviation from predicted in *Gt(l)* for the women was only almost significant when allowance was made for the lowered *TLC* in functional classes III+IV ($t=2.017$, significant if $t>2.014$). The men had a lower than normal *Gt(l)* whatever way the predicted normal value was calculated. Significantly few class III+IV subjects had values 100 per cent or more of predicted when calculating in this manner except when the predicted *Cdyn(l)* was based on the observed *VC*.

The *TLC*, *VC*, *MEF*25% and *Cdyn(l)*

tended to drop with increasing functional disability, and analysis of variance showed significant differences This held for *Cdyn(l)*, however, only when the predicted values were based on the predicted *VC* in the men, and the predicted *TLC* in the women

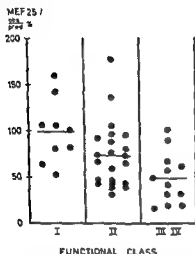


Fig 12 Relationship between maximum expiratory flow at 25 per cent of vital capacity ($MEF\,25\%$) and functional class in men and women with rheumatic valvular disease

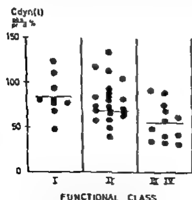


Fig 13 Relationship between dynamic compliance (*Cdyn(l)*) and functional class in men and women with rheumatic valvular disease Predicted values based on predicted values for *VC*

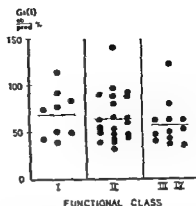


Fig 14 Relationship between inspiratory lung conductance (*Gi(l)*) and functional class in men and women with rheumatic valvular disease Predicted values based on predicted values for *VC* (men) and *TLC* (women)

Study of the effect of the duration of dyspnea on the pulmonary function in functional class II showed no tendency to worsening of the values for any of the variables of respiratory function with increasing duration of dyspnea

Comments

The observations in this study correspond to those in several previous studies but most of these have not considered the effect that chronic bronchitis might have had on their results for lung mechanics In this study no significant differences emerged between the heart patients with and without chronic bronchitis Cases of advanced chronic bronchitis however are known to show much lower values for *Gi(l)* for example than the ones registered in the present patients

Peabody & Wentworth (1917) found that an increase in dyspnea was correlated with a decrease in *VC* Hewlett s (1924) 900 men and women with heart disease had a *VC* between 25 and 130 per cent

of the normal and his patients with an enlarged heart had a *VC* only 66 1 per cent of normal, against 77 4 per cent of normal in the group as a whole Twenty one out of Gardam's (1950) 321 patients aged 20–42 with rheumatic heart disease in childhood had a lowered *VC*, of these, 3 belonged to functional class I 14 to class II and 4 to class III His cases with a lowered *VC* often had an enlarged heart

Frank *et al* (1953) made a study much like the present one of 62 patients with rheumatic heart disease and without a prominent background of broncho pulmonary infection In this series *VC* was 98 94 91 and 89 per cent of the predicted normal in functional classes from I to IV Unlike Altschule & Zamcheck (1944) who stated that *VC* rose surprisingly little when pleural transudate was withdrawn they noted that patients with hydrothorax had a lower *TLC* and *VC* than other patients in the same functional class and likewise patients with ascites The last observation led to a study by Abelman *et al* (1954) of 8 cases of ascites due to heart disease, they found that treating the cases caused *VC* to rise significantly from 1 95 to 2 34 *L* in 7 of these 8 an average of 11 1 *L* of fluid were withdrawn The 8th case was treated with drugs this case showed the greatest improvement *VC* rising by 0 7 *L*

The *VC* also dropped with increasing functional disability in Verstraeten *et al*'s (1958) cases amounting to 97 87 and 66 per cent in classes II III and IV

Unlike the foregoing authors Palmer *et al* (1963) did not observe that *VC* fell with increasing functional disability but noted that it was lower than the predicted normal in mitral valvular disease

Granath's (1965) 57 patients with mitral stenosis had a *VC* 0 95 *L* under normal and the value did not rise significantly after operation though 40 of the 57 were assigned to lower classes afterwards

West *et al* (1953) said that the lung volumes remained normal in heart disease unless fluid was retained in the lung Thus their findings disagree with those in the present study, and with the results of several of the aforementioned studies on well compensated patients which showed a decrease in *TLC* and *VC* in these patients The difference in their results is apparently not explainable by their having selected subjects in another way or having used different methods

Though all the groups of patients in the present series had a high *RV* in relation to their low *TLC*, none showed a higher absolute *RV* than predicted Several other authors however, contend that *RV* is raised in heart patients Thus Binger's (1923) 12 patients with different heart diseases had an *RV* above the predicted normal on treatment it dropped in some cases Lundsgaard's (1923) compensated patients also had a higher *RV* than normal, while his decompensated ones had a lower *RV* In Frank *et al*'s (1953) series *RV* equalled 105 139, 152 and 129 per cent of the predicted normal in functional classes I II, III and IV, respectively They could not confirm Altschule & Zamcheck's (1944) observation that *RV* was particularly low among patients with pleural effusion, but they remarked that it was lower when ascites was present Verstraeten *et al* (1959) also reported that *RV* rose with increasing functional disability

West *et al* (1953) however, found a

normal RV in 18 cases of mitral stenosis in different functional classes, and Brown *et al* (1954) and Granath (1965) did the same in 17 and 57 similar cases, respectively This contradiction in results may be due at least partly to the use of different methods Thus Binger (1923) Lundsgaard (1923), Frank *et al* (1953) and West *et al* (1953) used oxygen-dilution methods, but the later authors abandoned these in favor of the more reliable helium dilution methods

FEV_1 tended to drop with increasing clinical disability in this study, but the increase in disability ran practically parallel with the drop in VC, and $FEV_1\%$ did not differ from the predicted normal for the age in any class

Frank *et al* (1953) observed that $FEV_1\%$ dropped consecutively from functional class I to IV, amounting to 84, 75 71 and 69 per cent, respectively, and Verstraeten *et al* (1958) got values of 73, 62 and 56 per cent in classes II, III and IV respectively Friedman Macias and Yu s (1959) figures for the same groups were 78 77 and 70 per cent In Aber s (1963) series of patients without bronchitis, $FEV_1\%$ was 78 74, 69 and 70 per cent in functional classes from I to IV

Frank *et al* s results may be explained by the rise in the median age of their patients from 24 years in class I to 46 years in class IV and to their containing cases of fluid retention The same applies to the cases studied by Verstraeten *et al* (1958)

As to the values for maximum expiratory flow neither PEF, $MEF50\%$ nor $MEF25\%$ were significantly correlated with the lung volumes in the men in this study either controls or heart patients

PEF was more strongly connected with VC in the women with heart disease in the present study than in the control women R^2 amounting to 0 55 and 0 29, respectively (tables 3 and 16)

The PEF is relatively unspecific and other factors than the resistance to air flow in expiration such as neuromuscular factors and the cooperative ability of the patients may affect the values No clear tendency to a decrease in PEF with increasing disability was forthcoming in the present study

$MEF50\%$, like PEF, was more strongly connected with VC in women with heart disease than in the control women R^2 amounting to 0 55 and 0 30 respectively (tables 3 and 16) Thus it may be that a drop in VC is partly responsible for the changes in PEF and $MEF50\%$ in heart disease

$MEF50\%$ should depend less on extrapulmonary factors than PEF The low values for $MEF50\%$ in the present patients and their tendency to drop with an increase in functional disability correspond with the findings of Palmer *et al* (1963) who determined the $MEF25$–75% according to the method of Leuallen and Fowler (1955) Aber (1963) tried to detect the presence of airway obstruction by calculating the ratio between the maximum mid inspiratory and mid expiratory flow rate he found no increase in the ratio in severe functional disability

$MEF25\%$ was also more strongly connected with VC in the women with heart disease than in the control women R^2 amounting to 0 45 and 0 26, respectively (tables 3 and 16)

The lower part of the flow volume curve

including *MEF*25% should depend even less than *PEF* and *MEF*50% on extra pulmonary factors, and as pointed out on page 25 it reflects more than they do the resistance of the peripheral airways Wood *et al*'s (1967) patients with mitral stenosis showed significantly lower *Vmax* values between 80 and 10 per cent of *VC* than a control group

The present study also showed decreasing values for *MEF*25% with advancing functional disability, more so than for *MEF*50%

As to *Cdyn(l)* in order to try to differentiate between the effects of the lung volume and tissue elasticity on the compliance the normal values in the present study were calculated both from the observed and predicted *VC* With the predicted *VC* the normal value was reckoned from the lung volume (and lung tissue mass) normal for the body size But it must be remembered that the lung volume may be diminished because of other structures particularly the heart taking up an abnormal amount of room and this calculation may therefore be based on a misleadingly high value for volume in cases of heart disease

The predicted values for *Cdyn(l)* obtained from the observed and predicted *VC* often differed Thus when the predicted *VC* was used *Cdyn(l)* was subnormal both in the women in functional class II and class III+IV and *Cdyn(l)* tended to drop with increasing functional disability while if the observed *VC* was used for prediction only the women in functional class III+IV had a lower than predicted *Cdyn(l)* On the other hand *Cdyn(l)* was significantly subnormal in the women as a whole even when the predicted value was reckoned from the observed *VC*

Christie & Meakins (1934) noted that cases of pulmonary congestion needed a greater change in pleural pressure to change the lung volume by 20 per cent of the *FRC* than normal cases, and less when they were treated Mead *et al* (1953) got a *Cdyn(l)* of 0 12 in their compensated cases of mitral stenosis and of 0 08 *L*/cm H_2O in decompensated cases and found that *Cdyn(l)* was positively correlated with *VC* in their altogether 11 cases Marshall *et al* (1954) also noted that the *Cdyn(l)* and *VC* were positively correlated in 25 patients with mitral valvular disease, and that the coefficient of elastic resistance was higher than normal Brown *et al* (1954) observed that the static elasticity was higher than normal in 17 cases with rheumatic heart disease, that this variable was strongly positively correlated with the inverted value for *VC*, and that the regression lines for normal and diseased subjects were essentially the same Others have also noted that *Cdyn(l)* is closely correlated with *VC* in heart patients (Turino, 1956 Saxton *et al*, 1956 Frank *et al* 1957 Nisell *et al* 1958 Verstraeten *et al* 1959)

As to pulmonary resistance or conductance Mead *et al* (1953) got results like those in the present study Thus the total pulmonary resistance amounted to 2 5± 1 4 cm H_2O/*LPS* in their 11 healthy subjects, but to 2 3±0 8 in 8 patients with mitral stenosis without clinically manifest pulmonary congestion and to 4 7 cm H_2O/*LPS* in 3 cases with pulmonary congestion Their groups did not differ clearly in their values for FEV_1 maximum voluntary ventilation or air velocity index

Brown *et al* (1954) also observed that the total pulmonary resistance was often increased in their group of heart patients mostly with rheumatic heart disease They concluded that this increase was due to increased resistance against the flow *of gas, not to tissue resistance and that* it was caused by edema in the bronchial walls, or fluid in the bronchial lumina They also discussed the possibility that some branches of the bronchi might be completely cut off by fluid in these cases making other regions raise their ventilation resulting in higher flows and higher resistance pressures Noting that some of their patients showed a normal resistance despite relatively pronounced dyspnea Brown *et al* concluded that dyspnea in heart disease stemmed more from a reduction in vital capacity and alterations in elastic properties than from an increase in resistance Their observations agreed well with Christie s (1953) graphs showing the elastic and nonelastic components of the work of breathing at rest and on exercise

Nisell *et al* (1958) and Palmer *et al* (1963) confirmed the foregoing observations of a high total pulmonary resistance in patients with mitral stenosis and a tendency to higher values with increasing functional disability

Turino & Fishman (1959) expressed a contrary opinion with respect to pulmonary resistance i e in the subject with pulmonary congestion the work done against air flow resistance is normal but the compliance is approximately three times less than normal ' Later in the same paper however they wrote When pulmonary congestion is complicated by frank pulmonary edema or cardiac asthma the air flow resistance further augments the work of breathing '

A recent study by Wood *et al* (1967) also showed an increased pulmonary resistance in mitral valvular disease, [illegible] 8±0 4 cm H_2O/LPS against 1 5±0 [illegible] in control *subjects* They *did not compare the resist*ance with functional disability

As the present control series indicate, it is important to consider the lung volumes in the case of conductance This was done in the present study by calculating the normal values for $G_l(l)$ from both the observed and predicted *VC* and *TLC* for men and women The low lung volumes cannot have been the sole reason for the reduction in conductance in the present series of men and women patients as a whole for the values were below normal even compared with the predicted values based on the observed lung volumes The slight lowering of the conductance with increasing functional disability noted in this study vanished on correction for the lung volumes and in no case was the trend significant thus these results do not support the observations of Nisell *et al* (1958) and Palmer *et al* (1963)

Lung mechanics in relation to pulmonary roentgenograms

It was possible to grade the pulmonary roentgenograms according to the method described on page 16 for 15 of the 17 men and for all 26 women with mitral and/or aortic valvular disease The following shows the amount of functional disability in these subjects grouped according to the appearance of their pulmonary roentgenograms

Roentgenographic classification	Functional class (N.Y.H.A.) I	II	III	IV	Total
0	4	6	1	—	11
1	2	5	1	—	8
2	2	8	5	—	15
3	—	1	1	—	2
4	1	1	2	1	5
Total	9	21	10	1	41

The figures show that the pulmonary changes tended to increase with an increase in functional disability, but the tendency is not consistent for example, the five cases with grade 4 pulmonary changes four of them women, are distributed over all four classes of functional disability

In order to get groups big enough for analysis the subjects with normal roentgenograms were pooled with the ones showing mild chronic congestion (0 and 1) and the ones with more advanced changes (2 3 and 4) collected in a second group The men with mild or no roentgenographic changes did not differ from the men with severe roentgenographic changes in $\bar{P}_{PA}$, $\bar{P}_{LA}$ or PVR but the women with severe roentgenographic changes showed significantly higher means than the other women the figures being as follows $\bar{P}_{PA}$ 18 8 against 34 [illegible] mm Hg ($P<0\ 005$) $\bar{P}_{LA}$ 13 [illegible] against 20 3 mm Hg ($P<0\ 02$) PVR 1 22 against 3 96 mm Hg (L/min) ($P<0\ 01$)

As seen from table 14 the predicted normal values for the different variables of lung function were slightly higher for the men with severe roentgenographic changes than for the men with mild or no changes This was because they were slightly taller on the average though they were of about the same average age

Consequently it was possible to compare the observed values directly without overestimating the differences between the groups The men with severe changes showed a lower FEV_1, $FEV_1\%$, $MEF25\%$ and $Cdyn(l)$ than the others The same differences emerged on comparison with the predicted values this is seen most clearly in the case of VC, FEV_1, PEF, $MEF50\%$ and $Cdyn(l)$

The values for lung volumes in the women with mild or no roentgenographic changes did not differ from the predicted normal values (table 15) The values for the other women were low for all the variables except the RV and PEF regardless of how the predicted values were calculated

The women with changes of grade 3 and 4 had higher values for $\bar{P}_{PA}$, $\bar{P}_{LA}$ and PVR than the grade 2 women, but did not differ in variables of lung mechanics

As seen from the top of page 55 the men and women with severe roentgenographic changes showed lower values for observed in per cent of predicted for TLC, VC $MEF25\%$ and $Cdyn(l)$ than the others The sign test revealed that the subjects with severe changes differed significantly from normal in all tested variables but that only $Cdyn(l)$ was significantly subnormal in the group with mild or no roentgenographic changes

To try to throw further light on the connection between roentgenographic changes in the lungs and the variables of respiratory mechanics, the observations in functional class II were subjected to further analysis the subjects in this class with mild or no roentgenographic changes being compared with the ones with more severe changes the two groups contained

	Roentgenographic findings						Signif of difference
	Grade 0–1 (n = 19)			Grade 2–4 (n = 22)			
	$\bar{x}$	$s_{\bar{x}}$	No with ≥ 100% of pred.	$\bar{x}$	$s_{\bar{x}}$	No with ≥ 100% of pred	$p<$
TLC	92.2	2.7	5	82.6	2.3	2	0.01
VC	89.6	3.2	5	75.6	2.4	0	0.01
*MEF*25%	85.5	8.7	4	62.4	7.3	[illegible]	0.05
Cdyn(l)[a]	85.7	5.5	5	64.4	4.3	1	0.005
Cdyn(l)	96.8	4.8	10	79.7	5.1	5	0.05
Gi(l)[a]	71.1	6.6	2	57.3	4.4	1	—
Gi(l)	77.1	6.1	3	63.9	3.7	3	—
Sign test $p < 0.05$ if			(4/15)[b]			(5/17)[b]	

[a] Predicted value based on predicted value for *TLC* or *VC*

[b] These figures give the proportions the sign test requires for significance at the 5 per cent level.

11 and 10 patients, respectively. The *TLC* in the two groups averaged 90 and 86 per cent of predicted, respectively, and the *VC* 89 and 80 per cent of predicted. These values do not differ significantly. *MEF*25% averaged 84 and 65 per cent of predicted, but nor do these figures differ significantly. *Gi(l)* on the other hand differed significantly in the two groups, averaging 83 per cent of predicted in the first group and 57 per cent in the second ($p<0.02$).

A similar analysis was done of the *VC* in the cases with a $\bar{P}_{PA}$ of 20–29 mm Hg, but it revealed no difference in *VC* between the cases with roentgenographic changes of grade 0–1 and of grade 2–4.

Comments

The roentgenographic appearance of the lungs may vary within relatively short intervals in patients with mitral stenosis without any concomitant change in functional class. In the present study the roentgen examination was performed soon before the other examinations with the patient in virtually the same condition.

Nisell *et al.* (1958), the only other authors who have made an analysis similar to the present one, did not detect any connection between the roentgenographic appearance of the lung and the compliance and resistance. The present study demonstrated differences in lung mechanics between those with mild and severe roentgenographic changes, but this may be because roentgenographic changes mostly run parallel with the clinical disablement and pulmonary vascular pressures. Thus the patients with roentgenographic changes of grade 0–1 in functional class II differed from the patients in the same functional class with changes of grade 2–4 in central hemodynamics, $\bar{P}_{PA}$ being 20 and 27 mm Hg, respectively.

Lung mechanics in relation to cardiac size and hemodynamic variables

The connection between the expressions for lung mechanics as dependent variables, and the roentgenographic heart volume*), (*RHV*), the mean pressure in the pulmonary artery ($\overline{P}_{PA}$), the mean pressure in the left atrium ($\overline{P}_{LA}$) and the pulmonary vascular resistance (*PVR*) as independent variables, was tested together with age and anthropometric variables by multiple regression analysis. Tables 16 and 17 show the significant relationships emerging from this analysis. Regressions are also given for anthropometric variables alone to make it possible to compare the determination coefficients.

The men were too few to expect many significant correlations but multiple regression analysis was done for them also and the results are given briefly after the results for the women.

For some variables of lung mechanics, the observed values in per cent of predicted were correlated with the hemodynamic variables, making it possible to combine the men and the women. The correlation coefficients are seen at the top of the next column with $p>0.05$ correlations given in brackets. The correlation coefficients are given instead of the determination coefficients to be able to show the sign.

	RHV	$\overline{P}_{PA}$	$\overline{P}_{LA}$	*PVR*
TLC	−0.42	−0.40	(−0.22)	−0.45
VC	−0.[illegible]	−0.48	−0.35	−0.49
MEF25%	(−0.03)	(−0.2[illegible])	−0.42	(−0.18)
Cdyn(l)[a]	−0.36	−0.41	(−0.2[illegible])	−0.43
Gt(l)[b]	(+0.06)	(+0.03)	(+0.07)	(+0.04)
Gt(l)[c]	+0.[illegible]	(+0.1[illegible])	(+0.08)	(+0.19)

[a] Predicted values based on predicted *VC*. When observed values for *VC* were used, no significant correlations were found.

[b] Predicted values based on predicted values for *TLC* (women) or *VC* (men).

[c] Predicted values based on observed values for *TLC* (women) or *VC* (men).

The values for lung mechanics were also compared with the predicted normal in the men and women with heart disease grouped according to the $\overline{P}_{PA}$, $\overline{P}_{LA}$ and the *PVR*, that is, into 3 groups for each of the three, so as to have each group contain as near as possible the same number of subjects and so that the values for the hemodynamic variable in each of the first groups were about the same as those generally accepted as normal for the resting state. Tables 18–23 show the results.

*) Standing position.

Lung volumes

TLC, which in the control series was only correlated (positively) with height, was also negatively correlated in the women patients with each of the variables *RHV*, $\overline{P}_{PA}$ and *PVR*, and also positively correlated with weight in the equation containing *RHV*. When *RHV* and $\overline{P}_{PA}$ or *RHV* and *PVR* were tested together in the regression analysis, no significant correlations were obtained for any of the three. The highest value for R^2 was obtained with the variables height, weight and *RHV*. *RHV* raised R^2 from 0.51 to 0.65. R^2 increased from 0.42 in the equation only including height to 0.54, and to 0.55 with $\overline{P}_{PA}$ or *PVR*. *RHV* was positively correlated with both $\overline{P}_{PA}$, $\overline{P}_{LA}$ and *PVR*, the correlation being strongest ($R^2=0.32$) for *PVR*.

In the men, TLC was only significantly correlated with height combined with PVR, and with PVR alone the first combination giving the higher value for R^2 TLC was not correlated with RHV $\overline{P}_{PA}$ or $\overline{P}_{LA}$ in the men

On analysis of the observed values in per cent of predicted for the men and women combined significant correlations appeared between TLC and RHV, $\overline{P}_{PA}$ and PVR as seen from page 56 and figs 15 and 16 The correlation coefficients were similar in size and it made no difference as far as the RHV was concerned whether or not correction was done for the body surface area Excluding the cases with a very high PVR [$\geq$8 mm Hg/(L/min)] did not affect the correlation coefficient for the connection between TLC and PVR The coefficient of regression on the other hand differed for the correlations obtained with and without these cases of a high PVR amounting to -0 62 and -0 19 respectively The observations were analogous for VC (fig 18)

RV was only positively correlated with age and TLC in both the men and women but TLC as mentioned depended on different hemodynamic variables

In the women FRC depended on the height but not on the age or any hemodynamic variable

VC in the women was negatively correlated with age and positively with height and weight and the regression equation with these three variables had a determination coefficient of 0 69, thus one much higher than in the control series TC was also negatively correlated with each of the variables RHV $\overline{P}_{PA}$ and PVR in the women Addition of these variables raised the R^2 to 0 80 0 77 and 0 76 respec

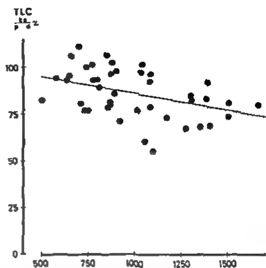


Fig 15 Relationship between total lung capacity (TLC) and roentgenographic heart volume (RHV) in men and women with rheumatic valvular disease Regression equation

$TLC\frac{obs}{pred}\%= -0\,0178\ RHV + 103\,9 \quad R=0\,42$

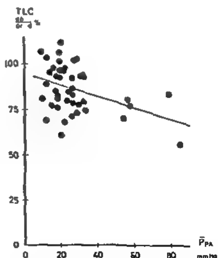


Fig 16 Relationship between total lung capacity (TLC) and mean pulmonary artery pressure ($\overline{P}_{PA}$) in men and women with rheumatic valvular disease Regression equation

$TLC\frac{obs}{pred}\%= -0\,316\ \overline{P}_{PA} + 94\ldots \quad R=0\,40$

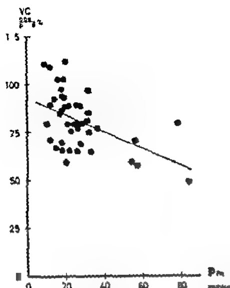


Fig 17 Relationship between vital capacity (VC) and mean pulmonary artery pressure ($\bar{P}_{PA}$) in men and women with rheumatic valvular disease Regression equation

$$VC \frac{obs}{pred} \% = -0.439 \quad \bar{P}_{PA} + 92.6 \qquad R = 0.48$$

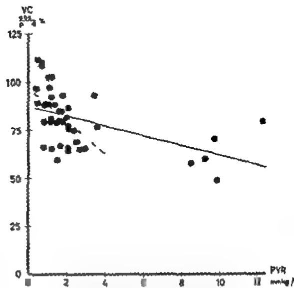


Fig 18 Relationship between vital capacity (VC) and pulmonary vascular resistance (PVR) in men and women with rheumatic valvular disease Regression equation for the whole series (solid line)

$$VC \frac{obs}{pred} \% = -2.57 \quad PVR + 87.4 \qquad R = 0.49$$

and when the ones with $PVR \geq 8.0$ mm Hg/(L/min) are excluded (broken line)

$$VC \frac{obs}{pred} \% = -8.00 \quad PVR + 96.7 \qquad R = 0.47$$

tively and lowered the RSD to about 0.40 L When RHV was tested together with $\bar{P}_{PA}$ or PVR in the equation with VC all three significant negative correlations between VC and the hemodynamic variables disappeared As mentioned RHV in the women was positively correlated with P_{PA} and PVR

In the men age together with height gave an R^2 of 0.41 in the equation with VC and adding $\bar{P}_{PA}$ and PVR raised R^2 to 0.61 and 0.66 respectively RHV did not significantly affect VC in the men

The coefficients for age were about twice as large in the heart series as in the control series both in the men and the women but the coefficients for height were the same

The regression analysis covering both men and women of the observed values for VC in per cent of predicted on the one hand and each of RHV $\bar{P}_{PA}$ $\bar{P}_{LA}$ and PVR, on the other showed significant connections in each case The correlation coefficients were of about the same size but highest for $\bar{P}_{PA}$ and PVR The two last correlations are shown in figs 17 and 18 The same was true of VC's correlation with PVR as for TLC's The coefficients of regression differed, depending on whether all the cases were included or only the ones with a PVR less than 4 mm Hg/(L/min) (fig 18)

FEV_1 in the women was negatively

correlated with age, positively with height and weight, and negatively with RHV. Their FEV_1 was also negatively correlated with age and positively with height and negatively with $\bar{P}_{PA}$ and $\bar{P}_{LA}$ respectively. The determination coefficients rose on addition of the hemodynamic variables from 0.71 to 0.77 for the regression with age, height and weight and from 0.54 to 0.62 and 0.64 for the two with age, height and $\bar{P}_{PA}$ and $\bar{P}_{LA}$, respectively. When RHV and $\bar{P}_{LA}$ were tested together with age and height, the coefficient for P_{LA} became almost significant, while the correlation with RHV disappeared.

In the men FEV_1 was negatively correlated with each of the variables $\bar{P}_{PA}$, P_{LA} and PVR but not with RHV. It was not significantly correlated with height in the men.

The regression of FEV_1 on VC did not reveal any significant correlations with age, hemodynamic variables or RHV either for men or women. As in the case of VC, the correlation with age was almost twice as strong as in the control series.

The only significant correlation in the case of FEV_1% was a negative correlation with $\bar{P}_{LA}$ in the men.

Subdividing the women according to their values for P_{PA} and P_{LA} showed a significantly low TLC and VC but not FEV_1 with $\bar{P}_{PA}$ over 30 mm Hg and with P_{LA} above 20 mm Hg, when FEV_1 was also significantly low. The women with a PVR of 1.5 to 2.4 mm Hg/(*L*/min) showed a lower TLC, VC and FEV_1 than normal. RV reckoned from the observed value for TLC was higher than normal in the women with the highest pressures and also those with the highest PVR.

The corresponding was true of the lung volumes in the men, but the deviations from normal were significant already in the middle groups and for TLC even for the groups with the lowest pressures or PVR. The groups with the highest pressures and highest PVR were too small to permit any conclusions.

Maximum expiratory flows

As seen from tables 16 and 17, PEF was not directly dependent on any hemodynamic variable and in the men not on any anthropometric variable or lung volume either. The best independent variable for the women was VC, which in turn depended on the hemodynamic variables and so PEF was indirectly dependent on these. The same applied to MEF 50%.

MEF25% in the women was negatively correlated with P_{PA} and $\bar{P}_{LA}$ in the regression with the height. On addition of these hemodynamic variables the determination coefficient rose from 0.25 to 0.32 and 0.36 respectively. MEF25% combined with age was also negatively correlated with P_{LA}, the R^2 amounting to 0.28. Like the other variables of $\dot{V}max$, it was relatively strongly correlated with VC.

The men showed no significant correlations between MEF 25% and the hemodynamic variables or the lung volumes but they showed the same tendencies as the women in this respect.

The observed values for MEF50% in per cent of predicted were tested against RHV, $\bar{P}_{PA}$, P_{LA} and PVR with both men and women included in the analysis (page 56). All that emerged was a negative correlation with $\bar{P}_{LA}$ (fig. 13).

Tables 18 to 23 show how the observed maximum expiratory flows differed from the predicted normal according to different

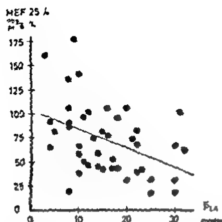


Fig 19 Relationship between maximum expiratory flow at 25 per cent of vital capacity ($MEF\,25\%$) and mean left atrial pressure ($\overline{P}_{LA}$) in men and women with rheumatic valvular disease Regression equation

$$MEF\,25\%\,\frac{obs}{pred}\% = -1\ 974\ \ \overline{P}_{LA} + 103\ 3$$

$R = 0\ 42$

pressures and PVR In the women PEF was lower than normal only with the highest $\overline{P}_{PA}$ and $\overline{P}_{LA}$ and with a PVR of 1 5 mm Hg/(L/min) or more $MEF\,50\%$ in the women was below normal reckoning from the predicted VC with a $\overline{P}_{PA}$ of 20 mm Hg or more a $\overline{P}_{LA}$ of 10 mm Hg or more and a PVR of 1 5 mm Hg/(L/min) or more $MEF\,25\%$ was also distinctly below normal in the groups with the highest pressures and PVR but it was also subnormal in the group with the lowest PVR

All the groups of men divided according to pressures showed subnormal values for PEF $MEF\,50\%$ tended to drop with a rise in pressure Thus it was subnormal with a $\overline{P}_{PA}$ of 19 mm Hg or less It was also subnormal with a PVR of 1 4 mm Hg (L min) or less As already pointed out some of the groups were extremely small $MEF\,25\%$ showed different tendencies depending on how the subgrouping was done it tended to drop on an increase in $\overline{P}_{PA}$, but was about the same in all three PVR subgroups

Indices for quantifying the slope of the flow volume curves were also worked out for the heart patients and they were also used in the regression analyses, and the means calculated for the different groups *In no case did any connection appear between these indices and the severity of* the heart disease or the hemodynamic conditions studied

Dynamic compliance

$Cdyn(l)$ was not fully significantly correlated with height alone in the women with heart disease ($t=1\ 86$ $t>2\ 00$ required for significance) but it was significantly correlated with height combined with PVR R^2 then becoming 0 35 It was negatively correlated with $\overline{P}_{PA}$ and PVR R amounting to 0 31 and 0 22, respectively It was positively correlated with TLC and VC R^2 amounting to 0 34 and 0 31 respectively These last two correlations were also forthcoming in the male series where R^2 amounted to 0 45 and 0 53 The women showed a tendency to $Cdyn(l)$ being negatively correlated with RHV, but the connection was not significant ($t=1\ 85$ $t>2\ 00$ required for significance)

Analysis of the men and women combined and using the observed values in per cent of predicted showed that If the predicted VC was used for calculating the predicted $Cdyn(l)$ $Cdyn(l)$ was negatively correlated with RHV and $\overline{P}_{PA}$ (fig 20 and 21) and with PVR, but when the observed value for VC was used all these three correlations disappeared

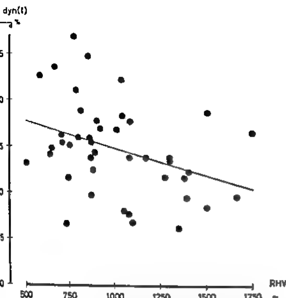


Fig. 20 Relationship between dynamic compliance ($Cdyn(l)$) and roentgenographic heart volume (RHV) in men and women with rheumatic valvular disease. Predicted value for $Cdyn(l)$ based on predicted value for VC. Regression equation:

$Cdyn(l)\frac{obs}{pred}\% = -0.0?93\ RHV + 103.?\ R = 0.36$

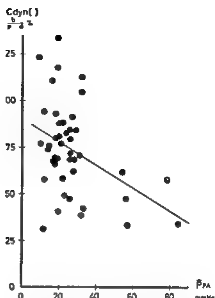


Fig. 21 Relationship between dynamic compliance ($Cdyn(l)$) and mean pulmonary artery pressure ($\bar{P}_{PA}$) in men and women with rheumatic valvular disease. Predicted value for $Cdyn(l)$ based on predicted value for VC. Regression equation:

$Cdyn(l)\frac{obs}{pred}\% = -0.6?3\ \bar{P}_{PA} + 90.5\ \ R\ 0.41$

In tables 18 to ?3 the compliance values are compared with the predicted normal values based on both the predicted and observed values for VC. Using the predicted VC $Cdyn(l)$ was lower than normal in most of the subgroups of women (except in subjects with a PVR of 1.4 or less and a PVR of 1.5–2.4).

Using the observed VC it was still subnormal in the groups with a $\bar{P}_{PA}$ of 30 mm Hg or more, a P_{LA} of 20 mm Hg or more, and a PVR of ?.5 mm Hg (L/min) or more, and also in the groups with a P_{PA} of 20–?9 and a $\bar{P}_{LA}$ of 0–9 mm Hg.

The men showed subnormal values only in the subgroups P_{PA} 20–?9 mm Hg, P_{LA} 10–19 mm Hg, PVR 1.5–2.4 and PVR 1.4 mm Hg/(L/min) or less when predicted $Cdyn(l)$ was based on predicted VC. The groups with the highest pressures and resistances contained only a few subjects each.

Conductance

$Gi(l)$ was positively correlated in the women with height, with TLC and with VC, the highest value for R (0.3?) occurring in the regression with TLC. It was also *positively* correlated with $\bar{P}_{PA}$ and PVR combined with TLC and also *positively* with PVR combined with VC.

In the men it was only *positively* correlated with RHV.

Comparing the observed values in per

cent of predicted with the different hemodynamic variables and the RHV in the men and women combined showed that $Gi(l)$ was positively correlated with RHV when the predicted values were based on observed values for TLC (women) or VC (men). Otherwise no correlations emerged no matter how the predicted $Gi(l)$ was calculated.

As seen from tables 18 to 23, $Gi(l)$ was below predicted in all the $\overline{P}_{PA}$, $\overline{P}_{LA}$ and PVR subgroups (though not all of them showed significant differences) when the predicted normal values were based on the predicted TLC and predicted VC. For some of the groups it was also significantly low when the normal values were based on the observed value for the lung volumes.

In relation to pulmonary blood volume

The pulmonary blood volume ranged between 320 and 710 ml in the 24 cases in which it was measured, the mean volume amounting to 535 ml. Calculated per square meter of body surface area according to the formula constructed by Forsberg (1964), the mean value was 314 ml/m² BSA, and the range 215 to 415 ml. Corrected for body surface area, the pulmonary blood volume was positively correlated with $\overline{P}_{LA}$ ($R = 0\ 48$) but not with $\overline{P}_{PA}$ or PVR. It was not correlated positively or negatively with the lung volumes, compliance or resistance, nor did it show any connection with the functional class or grade of roentgenographic changes.

Comments

The disorders of lung mechanics in rheumatic valvular disease have not been subjected to studies with multiple regression analysis by other authors, corrections for age and anthropometric variables having been made by other means.

Friedman *et al.* (1959) found that the observed VC in per cent of predicted was negatively correlated with $\overline{P}_{PA}$ ($R = -0\ 39$), total pulmonary vascular resistance ($R = -0\ 49$) and PVR ($R = -0\ 43$). They could not discover any correlation between RV and the hemodynamic variables nor any correlations between the hemodynamic variables and maximum breathing capacity.

In the present study, RV did not change significantly with the degree of functional disability or hemodynamic variables, so the decrease in TLC with worsening of these conditions must have been due to a decrease in VC.

VC was negatively correlated with each of the variables RHV, $\overline{P}_{PA}$, $\overline{P}_{LA}$ and PVR. When two of these independent variables were combined, the correlation lost its significance for both of them in the regression equations for women, and in the analysis where the men and women were combined, RHV increased with a rise in either the $\overline{P}_{PA}$, $\overline{P}_{LA}$ or the PVR. One can compare how much the hemodynamic variables and RHV affect VC by multiplying the mean value for the respective independent variable by its regression coefficient (b). This showed that RHV had almost twice the effect of $\overline{P}_{PA}$—0 78 versus 0 34 L—indicating that it affected the VC more than did the $\overline{P}_{PA}$, which might exert its effect via changes in the size of the heart.

In the regression with TLC the regression coefficient for RHV amounted to 0 000930 and in the one with VC it amounted to 0 000773. If the roentgeno

graphic measurement of the heart volume was completely accurate and the heart was the only structure which influenced the lung volumes in question, b should amount to 0 00100, i e, increasing the heart volume by a given amount should reduce TLC (and VC) by the same amount (RHV is given in ml in the regression equations but TLC and VC in L)

In the male series VC was not significantly negatively correlated with RHV but it was with $\overline{P}_{PA}$ and PVR, indicating that it was not only the enlargement of the heart in these men that lowered the VC But no definite conclusions can be drawn about this because the men were so few in number

In the majority of equations the absolute coefficients for age were about twice as large in the heart series as in the control series for both the men and women suggesting that VC drops more with age in cases of heart disease This is probably a function of the duration of the heart disease, for in this study the old patients had generally had their disease longer than the young ones It was not possible in the present series however to test the significance of the duration of the disease by comparing patients with the same $\overline{P}_{PA}$, $\overline{P}_{LA}$ and PVR but differing in the duration of their disease

The regression analysis for FEV_1 showed that it varied about the same with heart size and hemodynamic variables as did VC and so the variations can be attributed to the influences of these variables on VC Corroborating this, $FEV_1\%$ showed no connection with the heart size or hemodynamic variables except a negative correlation with $\overline{P}_{LA}$ in the men

$MEF25\%$ tended to drop with advancing functional disability more than the other values for $Vmax$, and in the women it also depended on the hemodynamic variables particularly $\overline{P}_{LA}$ $MEF25\%$, too depended more on VC in the women with heart disease than in the control women, R^2 amounting to 0 45 and 0 26 respectively These are apparently the first data published on the relationship between $Vmax$ and hemodynamic variables

The multiple regression analysis showed the same interdependence between $Cdyn(l)$ and lung volume as found for example, on study of the relation between compliance and functional disability Thus $Cdyn(l)$ was significantly correlated with $\overline{P}_{PA}$ and PVR but not when VC was included in the analysis

Other authors have reported a strong correlation between $Cdyn(l)$ and VC in heart patients (Mead *et al* 1953 Marshall *et al*, 1954 Brown *et al* 1954 Turino 1956 Saxton *et al* 1956 Frank *et al* 1957 Nisell *et al* 1958 Verstraeten *et al* 1958)

Saxton et al (1956) who did not correct $Cdyn(l)$ for lung volumes found no correlation between this variable and pulmonary vascular pressures pulmonary blood flow or pulmonary arteriolar resistance Pryor *et al* (1957) White *et al* (1958) and Saxton *et al* (1956) did not discover any correlation between pulmonary vascular pressures and compliance but they observed that $Cdyn(l)$ was subnormal in the groups with elevated pressures Verstraeten *et al* (1958) however found that lung elastance was positively correlated with the pulmonary wedge pressure

As mentioned, according to the regression analyses $Gi(l)$ was positively corre

lated with the heart volume and hemodynamic variables in the women patients. In reality it is more likely that the correlation was negative, for if heart disease affects the conductance at all, it should lower this property. Moreover, the decrease in $MEF\ 25\%$ with increasing $\bar{P}_{PA}$ and $\bar{P}_{LA}$ points to increasing time constants when the pressures increase, and this in combination with the decreasing $Cdyn(l)$ points to increasing resistance with increasing pulmonary vascular pressures. The positive correlation between $G_l(l)$ and various hemodynamic variables may be partly due to the effect of the lung volumes on $G_l(l)$. Doing multiple regression analysis with $G_l(l)$ as the dependent variable and TLC and P_{PA} as independent variables presupposes the unlikely situation that TLC can remain fixed when the $\bar{P}_{PA}$ increases. In reality TLC always decreases with increasing pressure or increasing heart size. This can be seen from equations 4 and 40 in table 16. If we assume that the $\bar{P}_{PA}$ increases up to its mean value in the series (=28.6 mm Hg), TLC will be decreased by 0.478 L. When this decrease in TLC is taken into consideration in the regression analysis (eq. 40), $G_l(l)$ will only increase by 0.04 LPS cm H_2O—a negligible amount. The same holds for equations 11 and 43 in table 16.

The men with heart disease showed no correlation between $G_l(l)$ and lung volumes but a positive correlation between $G_l(l)$ and RHV. This would indicate that still other factors affect the $G_l(l)$ in heart disease. As discussed on page 21, the heart may cause errors in values obtained for esophageal pressure. The heart acting as a mass load on the esophagus may decrease the amplitude of the flow resistive component of the pressure, and an enlarged heart may lead to erroneously high values for $G_l(l)$.

When the observed TLC was used for predicting $G_l(l)$, the observed/predicted $G_l(l)$ was positively correlated with RHV, whereas no significant correlation emerged when the predicted TLC was used for predicting $G_l(l)$.

As mentioned, the only significant correlation the pulmonary blood volume showed in this series was a positive one with $\bar{P}_{LA}$. Korsgren (1967), studying 74 cases of mitral stenosis covering all functional classes, found that this volume was strongly positively correlated both with TLC and VC. Besides this observation, no other results are available for comparison.

Static compliance and peripheral airway conductance

Three of the 10 patients in series (d) had symptoms pointing to chronic bronchitis. They had the same $Pst(l)$ as the other 7 and so they were not excluded for this study. In fig. 22 the values for $Pst(l)$ during expiration are plotted against the predicted TLC (table 24). As seen from this figure, the curves for the controls and patients intersect at FRC. The mean maximal elastic recoil pressure was the same in the patients and control subjects. The patients had a low mean TLC and VC (86.2±4.6 and 76.0±4.8 per cent of predicted, respectively).

Static compliance during expiration at the same value for FRC on which the $Cdyn(l)$ was based, was 0.122±0.020 in the 10 patients, which was significantly lower than predicted, both when the cal

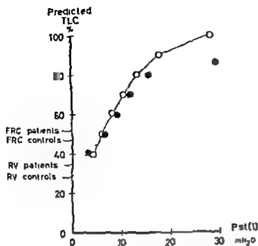


Fig 22 Average relationship between static recoil pressure (*Pst(l)*) and predicted total lung capacity (*TLC*) in 20 control women (solid line) and 10 women with rheumatic valvular disease (broken line)

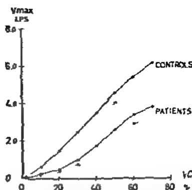


Fig 24 Average maximum expiratory flow volume curves before (solid lines) and after (broken lines) correction for alveolar gas compression in control subjects (20 women) and patients with rheumatic valvular disease (10 women)

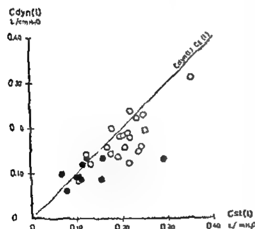


Fig 23 Relationship between static compliance (*Cst(l)*) and dynamic compliance (*Cdyn(l)*) in control subjects (unfilled circles) and patients with rheumatic valvular disease (filled circles)

culations were based on the observed ($p<0.005$) and the predicted *TLC* ($p<0.001$)

Fig 23 shows the relationship between *Cst(l)* and *Cdyn(l)* for the controls and patients Apart from one patient with mitral stenosis and chronic bronchitis the plot shows the same trend for both groups i e slightly lower values for *Cdyn(l)* than for *Cst(l)*

Table 25 gives the values for *Vmax* at 10 to 70 per cent of *VC* for the 10 women with mitral valvular disease and in fig 24 the data are compared with the values for the 20 control women As seen the alveolar gas compression had the same net effect on the mean curves in the control and diseased subjects

Gus was calculated for every ten per cent of *VC* The *Vmax* value corrected for alveolar gas compression was used for this so as to have *Vmax* and *Pst(l)* represent the same volume Table 26 gives the results for the patients as a whole

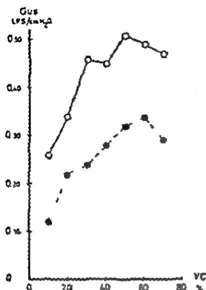


Fig 2 Relationship between peripheral airway or upstream conductance (*Gus*) and vital capacity in 10 women with rheumatic valvular disease (broken line) in relation to predicted normal values (solid line)

and subdivided into those with and without signs of chronic bronchitis The same is seen from fig 25 which also shows the predicted normal values yielded by the regression equations for the control series The patients as a whole differed significantly from the controls at 10 30 50 60 and 70 per cent of *VC* but only when the predicted value was calculated from the predicted *VC* and not when the bronchitic patients were excluded Two of the bronchitic patients had the same values as the nonbronchitic patients and the third had very low values for *Gus* (0 02 to 0 11 *LPS* cm H_2O) As seen from fig 25, the peripheral conductance curves had the same shape in the patients as in the controls

Comments

The observations on static recoil pressure in these 10 patients with mitral valvular disease are similar to those observed by Wood *et al* (1967) in their 23 patients with mitral valvular disease Wood *et al* also noted that the static pressure volume curve intersected the normal curve at *FRC*, and that *Cst(l)* at *FRC* was lower in the patients than in controls

Wood *et al* studied *Cdyn(l)* at two respiratory frequencies and found that it was independent of frequency in most cases of mitral stenosis The present study, in turn showed that *Cdyn(l)* and *Cst(l)* were similarly correlated in patients with mitral stenosis as in controls, the only exception being shown by a patient with chronic bronchitis and a very low *Gus*

The literature contains no data on the *Gus* in patients with rheumatic valvular disease As Mead *et al* (1967) pointed out the peripheral airway resistance theory does not hold so much for diseased lungs where some pathways may empty faster than others Inequality of pulmonary time constants does not seem to be a dominant feature of mitral stenosis however unless it is complicated by obstructive lung disease

Effect of diuretic therapy

All 7 subjects studied before and after diuretic therapy had been on digitalis for a long time and some of them had also been on peroral diuretic therapy At the time of this study none were taking any broncho dilating agent like isoprenaline or aminophylline

After examinations of lung function 5 of these subjects were given one or two

	Before therapy		After therapy		Diff	Signif of difference
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{d}$	$p<$
Age	54 4	5 3				
Height	163 1	2 0				
Weight	58 1	2 8	55 9	2 6	−2 2	0 001
RV	1 8	0 32	*			
FRC	2 53	0 36	2 60	0 31	+0 07	—
VC	2 49	0 37	2 65	0 33	+0 16	—
FEV_1	1 64	0 22	1 85	0 20	+0 21	—
PEF	3 66	0 63	4 34	0 71	+0 68	—
*MEF*50%	1 76	0 32	2 07	0 37	+0 31	—
*MEF*25%	0 48	0 08	0 60	0 14	+0 12	—
Cdyn(l)	0 125	0 017	0 130	0 0 0	+0 005	—
Gi(l)	0 3	0 08	0 61	0 14	+0 24	0 02
Ge(l)	0 25	0 05	0 42	0 05	+0 17	0 005
Pes(FRC)	6 0	1 [illegible]	8 2	1 2	+2 [illegible]	—

* *RV* only determined before therapy

doses of a mercury diuretic (Diurgin Astra) and ■ a peroral diuretic (Hygroton Geigy and Lasix Hoechst respectively) Two to six days later five days on the average the lung function was again examined

The results are seen at the top of this page (t test for paired comparison)

The subjects went down 0 6 to 2 5 kg in weight after taking the diuretic 2 2 kg on the average *RV* was only determined before the treatment and the values for *FRC* afterwards are calculated on the assumption that the *RV* remained the same though it is conceivable that this variable increased somewhat *VC* rose by 0 86 *L* in 1 case and less in 3 others and did not change significantly in the series as a whole FEV_1 and $FEV_1\%$ increased in 6 cases but not significantly in the group as a whole *PEF* rose in each case *MEF*50% in 4 cases and *MEF*25% in 3 but none of these rose significantly in the group as a whole due at least partly to the high s_d in some instances *Cdyn(l)* did not change but the respiratory rate rose after the treatment and the tidal volume fell slightly *Gi(l)* and *Ge(l)* rose after the treatment in each case without a significant change in the *FRC*

The end expiratory esophageal pressure on breathing at rest—*Pes(FRC)*— became more negative in 5 cases but did not change significantly in the group as a whole In one patient who had had pulmonary edema the day before *Pes(FRC)* fell from −3 ■ to −12 3 cm H_2O at the same time as *ERV* rose from 0 44 to 0 97 and all the values *improved distinctly*, *Gi(l)* for example rising from 0 31 to 0 74 *LPS*/cm H_2O Before the treatment the average *Gi(l)* was lower than normal but it rose to the predicted normal value after the treatment

Comments

The increase in $G_i(l)$ and $G_e(l)$ after the treatment is interesting for they were the only variables of lung mechanics that changed significantly in the examined cases. The increase in $G_i(l)$ cannot be explained by an increased TLC or VC. If this were the reason, to increase $G_i(l)$ from 0.37 to 0.61, TLC would have to increase by about 1.50 L according to equation 39 in table 16, but VC only increased by 0.16 L and RV could not have increased by so much as the difference between 1.50 and 0.16 L.

Cherniack *et al.* (1957) made about the same observations in their study of heart patients with orthopnea in the sitting and supine position: the index of elastic resistance rose slightly on a change from the sitting to the supine position, while the viscous resistance rose considerably, particularly in the expiratory phase. It is also interesting that one of their patients who could not lie flat, showed no increase in elastic resistance, but a large increase in viscous resistance. One cannot rely on measurements of the esophageal pressure in the supine position, but if the heart exerts any pressure this should reduce the amplitudes, and thus one should be apt to get too low values for resistance in the supine position.

Sharp *et al.* (1958), studying pulmonary edema, found that $Cdyn(l)$ rose significantly from 0.037 to 0.089 L/cm H_2O after treatment, and that $G_i(l)$ rose from 0.10 to 0.19 and $G_e(l)$ from 0.11 to 0.23 LPS/cm H_2O.

GENERAL DISCUSSION

The apparatus chosen for this study proved to be satisfactory for analyzing the mechanical properties of the lungs. The Servo-Spirometer had only a minute loading effect on the respiratory system and its frequency response was suitable for recording the volumes and flows at the respiratory rates used.

Earlier studies of normal subjects have shown that the esophageal pressure agrees well with the intrapleural pressure, especially when the method described by Milic-Emili *et al.* (1964) is used. The frequency response of the pressure recording system also proved to be satisfactory. The esophageal pressure method can apparently also be used for heart patients in the sitting position, but the values for conductance noted in the present patients indicate that it might give erroneously low amplitudes in these cases.

The results from the present study show that small control series of thirty to forty persons selected on correct principles can give just as much information as a large number of controls chosen at random. The sex differences observed—for example different regression coefficients for age—show that men and women should be grouped separately when analyzing lung volumes, maximum expiratory flows, *Cdyn(l)* and *Gi(l)*. It is not known if the sexes also differ in *Cst(l)* and *Gus*. But this cannot be excluded and, as most of the heart patients were women, only women were studied for *Cst(l)* and *Gus*.

Lung volumes. The results in the patients with rheumatic valvular disease agreed with those of most of the studies done previously. Thus *VC* was subnormal and dropped with increasing severity of the disease, both judging by the amount of functional disability, the appearance of pulmonary roentgenograms, the size of the heart and each of the hemodynamic variables, viz. pulmonary artery pressure, left atrial pressure and pulmonary vascular resistance.

The low *TLC* in the heart patients was due to their low *VC*, for they did not have a significantly subnormal *RV*, and the decrease in their *TLC* was proportional to the decrease in their *VC*. Earlier authors have assumed that the low *VC* in heart patients is due to changes in the lung parenchyma, and sometimes to abnormal amounts of fluid in the lung tissue or pleura. The present study showed that enlargement of the heart also lowers *VC* and *TLC*.

Elastic properties of the lungs. Compliance was subnormal in the present patients. Several other studies have shown the same. But not all of these say at what lung volume the compliance was measured. For values for compliance to give more information than what can already be obtained from the lung volumes, that is

in order to get an idea of the elastic properties of the lungs, the compliance must be corrected for the lung volume which gives the highest correlation coefficient in normal subjects In the present study this was *VC* But according to Frank *et al* (1956) the *lung volume* is not all that affects the value for compliance the *amount of lung tissue* also does so If this is true, correcting for the lung volume observed might give erroneously high compliance values in some cases This was borne out by a preliminary analysis of some of the patients included in the present investigation (Wilhelmsen, 1965) This analysis revealed that some of the patients had a specific compliance i e *Cdyn(l)*/observed *FRC* clearly in excess of normal Most of these patients had a larger heart than the other patients in the series Their *Cdyn(l)* values should obviously have been corrected for a higher *FRC* than observed

Analysis of values from the present controls showed that *Cdyn(l)* was a little more closely correlated with *VC* than with *FPC* but what has just been said about *FRC* also applies to *VC*

It was impossible to judge the effect of space occupying processes in the thorax on the connection between *Cdyn(l)* and *VC* from the present heart patients for enlargement of the heart and accumulation of fluid in the pleural cavity are generally combined with changes in the hemodynamic variables Moreover pleural transudate and pneumothorax may each cause atelectasis of the lung tissue making it hard to judge how much the space-occupying process itself affects compliance Nor could multiple regression analysis which keeps different variables constant by mathematical means, give any information on this, because the independent variables are dependent on each other

Judging by the results in this study the lowered *Cdyn(l)* in rheumatic valvular disease is not only due to the lowered lung volumes Thus it was lower than predicted in the series as a whole even when the predicted value was based on the observed *VC*

Flow resistive properties of the lungs The results in the control series showed that *Gi(l)* depended on the lung volumes like other expressions for the flow resistive properties of the lungs, for example, maximum expiratory flows It apparently does not drop in proportion to a drop in *TLC* or *VC* when something happens to reduce the lung volume, such as the heart becoming enlarged It would seem, therefore that it is best to relate *Gi(l)* in heart patients to the predicted normal value for the lung volume

Regardless of whether the predicted or observed volume was used *Gi(l)* was abnormally low in the whole series of patients with rheumatic valvular disease and it reacted more to diuretic therapy than did *Cdyn(l)*

The reduced slope of the maximum expiratory flow volume curve in rheumatic valvular disease points to an increase in the time constants of the lungs—*Re(l)* *Cdyn(l)* As the present patients studied for this feature had a low *Cdyn(l)* they must not only have had an abnormally high resistance but a resistance even higher than necessary to make up for the lowering of *Cdyn(l)* The tendency to low values for *Gus* noted in these patients indicates that the high flow

resistance was at least partly due to a reduction in the caliber of the peripheral airways

Anatomic and functional considerations The results of examining the lung mechanics suggest that disorders in the elasticity of the lungs rest on different anatomic conditions than disorder in resistance to airflow and that the two properties react differently to different factors Thus *Cdyn(l)* increased only negligibly on diuretic therapy compared with *Gt(l)* and *Ge(l)* which increased significantly

Disorders in the elasticity of the lung tissue may be due to alveolar *fibrosis* (Curti *et al* 1953 and others) vascular lesions (Parker & Weiss 1936 Gough 1955) *interstitial and alveolar edema* (Hayek 1953) pulmonary congestion or any combination of these

While it is obvious that alveolar fibrosis may lower compliance in analogy with the conditions in lung fibrosis of other kinds severe alterations in the precapillary vessels may not necessarily do so Thus Korsgren Bjure Wilhelmsen & Varnauskas (unpublished study) found that *Cdyn(l)* and *VC* were normal in 5 out of 7 cases of multiple pulmonary embolism with severe pulmonary hypertension but a normal pressure in the left atrium

Pulmonary venous hypertension on the other hand seems to affect the elastic properties of the lungs Compliance for example is greatly reduced in pulmonary edema Thus Sharp *et al* (1958) found that *Cdyn(l)* averaged 22 per cent of normal in pulmonary edema and 54 per cent after treatment Buhlman *et al* (1959) and Sharp *et al* (1961) also found greatly reduced values in pulmonary edema One of the reasons for the reduction in compliance is a change in the alveolar surface tension (Brown 1957, Said Avery Davis Banerjee & El Gohary 1965)

As mentioned, it has been found that the compliance decreases on acute pulmonary congestion induced by increasing the total blood volume and raising the pressures in the pulmonary circulation by various means Bondurant *et al* (1960) demonstrated in experiments with a *g* suit however that the esophageal pressure may react to a number of external factors and thus that some experimenters have probably overestimated the part played by the congestion in the decrease in compliance they noted On the other hand even the directly measured intrapleural pressure increases on pulmonary congestion (Christie & Meakins 1934) It has also been shown that *Cdyn(l)* and *Cst(l)* fall on induced congestion even when the esophageal pressure at *FRC* remains unchanged Thus Giuntini *et al* (1966) reported that the *Cdyn(l)/FRC* ratio dropped 18 per cent and the *Cst(l)* at *FRC* dropped 15 per cent after the infusion of 1000–1500 ml of Macrodex in 5 cases

Several authors have noted that the compliance drops in patients with mitral stenosis when they exercise (Marshall *et al* 1954 Hayward & Knott 1955 Nisell *et al* 1958) Pryor *et al* (1957) observed the same but they found that when patients with severe mitral stenosis had an extremely low *Cdyn(l)* at rest it did not decrease much more on exercise They concluded that the stiffness of these patients' lungs was primarily due to changes in their lung parenchyma, not to increased vascular pressure

Borst *et al* (1957) observed that the compliance fell very little in dogs on only a rise in the pulmonary artery pressure Frank (1959) found that the effect of acute pulmonary congestion in cats varied with the lung volumes that the recoiling force *Pst(l)* was below normal at low lung volumes normal at about *FRC* but above normal at high lung volumes This observation was supported by the present study and in Wood *et al* s (1967) study of chronic pulmonary congestion. The observation that *Pst(l)* is subnormal at lung volumes under *FRC* bears out the early observations of von Basch (1891) who gave the name Lungenstarre" to the lung vessels erective effect on the tissue which he thought was caused by increased pulmonary venous pressure

Cook *et al* (1959) detected that the drop in compliance depended on the duration of the pulmonary congestion being only inconsiderable at first but dropping to as much as 78 per cent under the pre-experimental level after the congestion had been present for some time Remarking the presence of static hysteresis in their cases they said that this pointed to a change in alveolar surface tension probably due to fluid entering the alveoli They also discovered on distending edematous lungs over the region for the tidal volume that the overall compliance at a distending pressure of 30 cm H_2O was not much less than that of normal lungs and pointed out that this further underlined the significance of the surface tension.

Levine *et al* (1965) believed that the best way to determine whether pulmonary edema was present was to measure the pulmonary extravascular fluid volume with tritium They observed that on increased left atrial pressure and pulmonary engorgement by which they meant an increase in the pulmonary blood volume over the control value by once the standard deviation the compliance dropped 41 per cent from the pre experimental level while the *FRC* dropped 14 per cent on pulmonary edema they dropped 75 and 17 per cent respectively

The conditions in the interalveolar spaces are probably of great importance to the elasticity of the lungs and also to the resistance to airflow The pressure in these spaces is probably below that in the surrounding alveoli and bronchi (Howell Permutt & Riley 1961) In such a case a rise in the pulmonary venous pressure might distend the capillaries and constrict the bronchioli in these spaces stiffening the lung tissue at least at lung volumes over *FRC* and increasing the pulmonary blood volume But while the pulmonary blood volume was positively correlated with the pressure in the left atrium in the present study regression analysis did not reveal a significant negative correlation between it and *Cdyn(l)*

Varnauskas & Korsgren (1967) observed that increasing the plasma volume and the left atrial pressure did not change the pulmonary blood volume significantly and Wilhelmsen & Varnauskas (1967) observed that it caused a shift to the right in the static compliance curve of the lungs Even if one cannot detect any increase in the pulmonary blood volume in experiments of this kind it does not rule out the possibility of the capillary volume having increased, perhaps because of the blood being redistributed in some way among the different compartments of the pulmonary vascular bed

The extravascular fluid volume of the lungs generally remains normal after the infusion of Rheomacrodex (Varnauskas & Korsgren, 1967) but should it increase it probably increases mostly in the inter alveolar spaces (Hayek 1953) That there is an abnormal accumulation of fluid in these spaces in rheumatic valvular disease is supported by McCredie s (1967) observations

As mentioned on page 68 Sharp *et al* (1958) noted that the conductance dropped during pulmonary edema Intrabronchial edema might be one of the reasons for this That still other mechanisms might be responsible is indicated by the results in the present study both in the main patient series and in the patients studied before and after diuretic therapy

The diameter of the bronchioles may be decreased by distension of the venous plexus in their walls This plexus drains off the blood from the center of the pulmonary lobules while the septal veins drain off the rest The septal veins get blood from the peribronchial plexus at the prelobular level (Robertsson 1965) Hence an increase in the pulmonary venous pressure may be transmitted to the peribronchial plexus causing swelling of the bronchial mucosa and narrowing of the bronchial lumen

Levine *et al* s (1965) animal experiments support this explanation for the increase in bronchial resistance They found that $G_t(l)$ and $G_e(l)$ dropped on pulmonary engorgement though pulmonary engorgement was not associated with a rise in the extravascular fluid volume

As mentioned before the state of the interalveolar spaces probably also affects the resistance to flow West Dollery & Heard (1965) found support for this in experiments on isolated dog lungs stating Sometimes the perivascular space was ballooned out and the vessels compressed flat Bronchi and bronchioles were sometimes tightly constricted so that the mucosa was corrugated and the lumen small or obliterated It must be remembered however, that the conditions may be different *in vivo*

No correlation emerged between the pulmonary blood volume and $G_t(l)$ in the present study The extravascular fluid was not measured but as mentioned McCredie (1967) found that it increased on a rise in the left atrial pressure

The abnormal values for resistance to flow obtained in the patients with rheumatic heart disease examined in the present study and on experimental increase in the volume of plasma (Wilhelmsen & Varnauskas 1967) were probably due to changes in the interalveolar spaces and the peribronchial venous plexuses That the flow resistance may change without the compliance changing is clear from the results before and after diuretic therapy

Lung mechanics in relation to symptoms A great amount of interest has been attached to the relationship between lung mechanics and dyspnea Dyspnea is generally experienced on exertion whereas most studies of lung mechanics the present included have been made on subjects at rest It is already obvious from the resting values however that persons with rheumatic valvular disease have smaller volumes and flows in reserve than normal persons of the same size The esophageal pressure deflects more for a given variation in lung volume at rest and is even

more abnormal on exercise (Marshall *et al*, 1954 Hayward & Knott, 1955, Pryor *et al*, 1957, Nisell *et al*, 1958) Moreover, these patients often ventilate their lungs more at rest than on exercise compared with controls and so for this reason, too, need more respiratory work on exercise than do healthy persons (Peabody *et al*, 1917, Barr & Peters, 1920 Harrison *et al*, 1931 Espersen 1941, McIntosh *et al*, 1958 Stock & Kennedy, 1959, Gazetopoulos *et al*, 1966)

Chermiak *et al* (1957) observed that $G_1(l)$ fell much more than $Cst(l)$ on a change from the sitting to the supine position in heart patients and concluded that the decrease in $G_1(l)$ was the foremost reason for the increased dyspnea in the supine position The values obtained before and after diuretic therapy in the present study bore out this relationship between conductance and dyspnea

The dyspnea in rheumatic valvular disease is probably not only attributable to one factor Several of the physiologic variables involved are interdependent As Palmer *et al* (1963) also pointed out patients in functional class IV, for example, ventilate much more on exercise, because of their reduced diffusion capacity, limited cardiac output and the abnormal relationship they show between ventilation and perfusion Ventilation demands more respiratory work for them than for controls, and consumes more oxygen because of low compliance and conductance

The increased tendency to cough and expectoration in rheumatic heart disease can be explained by the swelling and edema in the bronchial mucosa and perhaps by an increased secretion from the mucosa Persons with constricted bronchial lumens and poor drainage facilities probably run a greater risk of bronchial infection than others

Irritating substances like tobacco smoke may have a more powerful effect on the conductance of the bronchi in rheumatic heart disease than they have in normal persons because of the already reduced diameter of the bronchi in this disease Whether the mucous membrane is more sensitive to irritants in subjects with this disease than in normal subjects is not known

Persons with rheumatic valvular disease suffer more often than others from wheezing especially, as mentioned on page 44 on exertion This cannot be only because of the increased expiratory airflow during exertion, as wheezing is not particularly common on forced expiration at rest It is conceivable, however, that the increase in pulmonary venous pressure during work leads to greater swelling of the bronchial mucosa and narrowing of the bronchial diameter It is true that Nisell *et al* (1958) reported that the conductance rose on exertion but this has not yet been confirmed It is more complicated to study lung mechanics during exercise than at rest It requires apparatus with a higher frequency response than for investigations at rest and it is also hard to compare conductance (and dynamic compliance) during rest and exercise for *FRC* is often different during rest than on exercise

SUMMARY

The object of the present study was to analyze in a series of patients with rheumatic valvular disease how the lung mechanics including lung volumes maximum expiratory flows compliance and conductance were related to symptoms appearance of pulmonary roentgenograms roentgenographic heart volume pulmonary vascular pressures and resistance and pulmonary blood volume

Subjects Sixty five patients (21 men and 44 women) with rheumatic valvular disease mainly mitral stenosis of different orders of severity aged 18–71 were studied The results from a main patient series consisting of 20 men and 31 women were compared with those from 72 control subjects (33 men and 39 women) without cardiorespiratory signs and symptoms aged 18 to 63 This control series was not selected at random but their results were checked against and found to agree with those from a series of 192 men also lacking cardiorespiratory signs and symptoms coming from a series of 875 randomly selected men aged 50 Analysis showed only minor differences between the controls and patients in smoking habits

Ten women with mitral valvular disease aged 29 to 71 and 20 control women aged 18 to 71 were studied for static compliance and peripheral airway resistance

Finally 1 man and 6 women patients aged 27 to 70 were studied before and after diuretic therapy

Methods A Servo Spirometer was used for measuring all the respiratory volumes and flows except the residual volume which was measured with the helium dilution method

Testing the Servo Spirometer for leakage sensitivity of the pressure transducer linearity of the volume and flow recording frequency response and simultaneity of the flow and volume signals proved that it was satisfactory for testing lung mechanics at rest

The esophageal pressure was measured with a 10 cm long rubber balloon with a perimeter of 35 mm containing 0 2 ml of air placed in the middle third of the esophagus The frequency response of this pressure recording system proved to be satisfactory

The respiratory variables were recorded with a sensitive system consisting of a Honeywell Visicorder and a Tektronix oscilloscope

All the subjects were examined in the sitting position They were examined for vital capacity (*VC*) forced expiratory volume per second (FEV_1) peak expiratory flow (*PEF*) maximum expiratory flow at 50 per cent of *VC* (*MEF* 50%) and maximum expiratory flow with 25 per cent of *VC* plus residual volume (*RV*) left in the lungs (*MEF* 25%) The highest of three values obtained for these variables was used for the subsequent calculations Study of the values from 20 controls and

20 patients revealed a difference of 1 4 and 1 9 per cent between the highest and next highest values for VC, and the corresponding figures for FEV_1 were 1 2 and 6 [illegible] for PEF [illegible] 4 and 10 5, for MEF 50% 12 [illegible] and 12 5, and for MEF 25% 12 7 and 12 [illegible] per cent

The effect of alveolar gas compression on the flow volume curves was determined in 20 controls and 10 patients and likewise the peripheral airway or upstream conductance (Gus)

The dynamic lung compliance $Cdyn(l)$, was examined in all the subjects and static lung compliance, $Cst(l)$ in 30 The error of a single determination of $Cdyn(l)$ proved to be 3 6 per cent in both controls and patients

The total pulmonary conductance $Gt(l)$, was calculated as the ratio between the flow $\dot{V}$ at 0 5 LPS, and the resistive component of esophageal pressure $Pres$ The error of a single determination of this value proved to be 18 7 per cent in controls and 15 4 per cent in patients

The data from the controls and patients were fed into a computer and among other things subjected to multiple regression analysis It was possible to use multiple regression analysis for studying the effect on the lung mechanics of the hemodynamic variables and the size of the heart as these are continuous variables When the absolute values for the variables were used for the statistical analysis, the men and women had to be separated but when the quotient consisting of the observed/predicted value was used the men and women could be combined

Results in control subjects In agreement with the results of previous studies VC and FEV_1 increased with increasing height and decreased with increasing age, PEF and $MEF50\%$ decreased with increasing age, and $MEF25\%$ decreased with increasing age, and the maximum expiratory flow volume curve became increasingly convex to the volume axis with increasing age $Cdyn(l)$ was positively correlated with age in combination with VC, and the same applied to $Cst(l)$ but the correlation between $Cst(l)$ and TLC gave a higher determination coefficient (R^2) But static recoil pressure, $Pst(l)$, was negatively correlated with age only at 100 per cent of TLC This may have been because $Cdyn(l)$ and $Cst(l)$ are calculated from differently inclining parts of the pressure volume curve in young and old subjects, due to the increase in FRC with increasing age $Gt(l)$ was positively correlated with VC in the men and with TLC FRC and VC in the women At 10 20 and 40 per cent of VC, Gus was negatively correlated with age At 50, 60 and 70 per cent it was positively correlated with VC TLC and age separately VC giving the highest value for R^2

The regression equations showing the highest R^2s in the control series were used for calculating the predicted normal values for comparison with the values from the patients

Results in patients with rheumatic valvular disease Cough, sputum and wheezing were more common among the patients with heart disease than in the standard population and were more common in the patients with high pulmonary arterial mean pressure ($\bar{P}_{PA}$), left atrial pressure ($\bar{P}_{LA}$), and pulmonary vascular resistance (PVR) than in the other patients

The main patient series was divided into ones with and without chronic

bronchitis i.e. cough and sputum at least three months a year. Then the patients with no symptoms of chronic bronchitis were divided (a) into different functional classes, (b) according to the observations in pulmonary roentgenograms and (c) according to the size of the heart and different hemodynamic variables. The significance of the pulmonary blood volume was also studied. Slightly different results were obtained for the different expressions of lung mechanics depending on whether the absolute or relative values were used. Likewise the results sometimes differed depending on whether the predicted normal values were based on the predicted or observed lung volumes. The results obtained from analysis of the combined sexes were briefly as follows.

With but few exceptions *TLC*, *VC*, *PEF*, *MEF50%*, *MEF25%*, *Cdyn(l)* and *Gi(l)* were lower than the predicted normal in the patients both with and without chronic bronchitis.

Analysis of the patients without chronic bronchitis revealed that:

(a) *TLC*, *VC*, *MEF25%* and *Cdyn(l)* dropped significantly with increasing functional disability.

(b) The pulmonary roentgenograms tended to grow more and more abnormal with increasing functional disability but not consistently. The patients with severe roentgenographic lesions had a lower *TLC*, *VC*, *MEF25%* and *Cdyn(l)* than those with mild lesions. Patients in functional class II and with severe roentgenographic changes had a lower *Gi(l)* than those with slight lesions in the same functional class.

(c) *TLC* was negatively correlated with *RHV*, $\bar{P}_{PA}$ and *PVR*. *VC* was negatively correlated with *RHV*, $\bar{P}_{PA}$, $\bar{P}_{LA}$ and *PVR*. The only significant correlation between *MEF25%* on the one hand, and *RHV* and the hemodynamic variables on the other was a negative one with $\bar{P}_{LA}$. *Cdyn(l)* was negatively correlated with *RHV*, $\bar{P}_{PA}$ and *PVR*, but only when the predicted value was based on the predicted value for *VC*. *Gi(l)* was not correlated with any of the hemodynamic variables but was positively correlated with *RHV* when the predicted value was based on the observed *VC* (men) and *TLC* (women). This correlation may have been due to *Gi(l)* being dependent on the lung volume and possibly to the heart acting as a mass load on the esophagus.

The pulmonary blood volume was not correlated positively or negatively with the lung volumes, compliance or conductance, nor did it show any connection with the functional class or grade of roentgenographic changes, but it was positively correlated with $\bar{P}_{LA}$.

In the special study of the 10 women with mitral valvular disease the curve for *Pst(l)* plotted against predicted *TLC* intersected the same curve for controls at *FRC*. The static recoil for the same women was higher than normal above *FRC* but lower than normal below *FRC*. *Cst(l)* at a normal *FRC* was lower than normal whether based on the observed or predicted *TLC*. *Cdyn(l)* was slightly lower than *Cst(l)* in both the controls and the 10 patients, each showing about the same relationship between these expressions. *Gaw* was lower than normal but most of the significant differences disappeared when the 3 patients with chronic bronchitis were excluded.

The 7 patients examined before and after one or two doses of a diuretic went down 2 2 Kg in weight on the average after the treatment Their $Gi(l)$ and $Ge(l)$ increased significantly but the other values for lung mechanics remained essentially unchanged Thus the increased conductance could not be explained by an increase in the lung volumes after the therapy

The results of this study indicated that the compliance of the lungs is reduced in rheumatic valvular disease, both because of decreased lung volumes and because of reduction in the elasticity of the lung tissue The reduction in elasticity may be due to vascular lesions and alveolar edema but most likely it is mainly due to interstitial edema and pulmonary congestion and possibly also to alveolar fibrosis

The lowered conductance is probably the result of swelling of the bronchial mucosa and structural changes in the intra alveolar spaces

The tendency to dyspnea in rheumatic valvular disease is probably due to the lungs needing more ventilation because of the lowered diffusion capacity, lowered cardiac output and disorder in ventilation perfusion accompanying this disease The patients need to do more respiratory work than normal because the compliance and conductance are lowered The tendency to wheezing in rheumatic valvular disease might be explained by the lowered bronchial conductance which seems to heighten the risk of bronchial infection

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REFERENCES

1 Abelmann, W H, Frank, N R, Gaensler, E A & Cugell, D W Effects of abdominal distention by ascites on lung volumes and ventilation Arch Int Med. 93 528 1954

2 Aber, C P "Bronchitis" in patients with mitral stenosis. Thorax 18 50 1963

3 Alsever, J B & Levine S A The immediate effect of mercurial diuretics on the vital capacity of the lungs Amer Heart J 15 201 1938

4 Altschule M D Zamcheck, N & Iglauer, A The lung volume and its subdivisions in the upright and recumbent positions in patients with congestive failure Pulmonary factors in the genesis of orthopnea J clin Invest 22 805 1943

5 Altschule, M D & Zamcheck N The effects of pleural effusion on respiration and circulation in man. J clin Invest 23 325 1944

6 Arnold F Ueber die Athmungsgrosse des Menschen J C B Mohr Heidelberg 1855

7 Attinger E [illegible] Goldstein M M & Segal M S The mechanics of breathing in normal subjects and in patients with cardiopulmonary disease Ann. intern. Med [illegible] 1269 1958

8 Attinger, E O Monroe R G & Segal, M S The mechanics of breathing in different body positions I In normal subjects. J clin Invest 35 904 1956

9 Barr, D P & Peters J P Jr Studies of the respiratory mechanism in cardiac dyspnea III The effective ventilation in cardiac dyspnea Amer J Physiol 54 345 1920

10 Bartlett R G Jr Pulmonary function evaluation in air and space flight Aerospace Med 32 685, 1961

11 Basch [illegible] S K von Klinische und experimentelle Studien A Hirschwald Berlin 1891

12 Bayliss L F & Robertson, G W Viscoelastic properties of the lungs Quart J exp Physiol 29 27 1939

13 Berglund, E Birath, G Bjure J Grimby G Kjellmer I Sandqvist L & Soderholm, B Spirometric studies in normal subjects I Acta med scand 173 185 1963

14 Binger, C A The lung volume in heart disease J exp Med 38 445 1923

15 Birath, [illegible] Kjellmer I & Sandqvist L Spirometric studies in normal subjects II Ventilatory capacity tests in adults Acta med scand 173 193 1963

16 Bittorf A & Forschbach J Untersuchungen uber die Lungenfullung bei Krankheiten. Z klin Med 70 474 1910

17 Bjure J Spirometric studies in normal subjects IV Ventilatory capacities in healthy children 7–17 years of age Acta paediat (Uppsala) 52 232 1963

18 Blair, E & Hickam, J [illegible] The effect of change in body position on lung volume and intrapulmonary gas mixing in normal subjects J clin Invest 34 383 1955

19 Bondurant [illegible] Hickam J B & Isley J K Pulmonary and circulatory effects of acute pulmonary vascular engorgement in normal subjects J clin. Invest 36 59 1957

20 BONDURANT S, MEAD J & COOK C D A re evaluation of effects of acute central congestion on pulmonary compliance in normal subjects J appl Physiol *15* 875 1960

21 BORST H G BERGLUND E WHITTENBERGER J L, MEAD J McGREGOR M & COLLIER C The effect of pulmonary vascular pressures on the mechanical properties of the lungs of anesthetized dogs J clin Invest *36* 1680 1957

22 BRANSCOMB, B The application of the respiratory flow volume loop in epidemiologic surveys Amer Rev resp Dis *86* 697 1962

23 BRENNER O Pathology of the vessels of the pulmonary circulation Arch intern Med *56* 211 1935

24 BRISCOE W A & DUBOIS A B The relationship between airway resistance airway conductance and lung volume in subjects of different age and body size J clin Invest *37* 1279 1958

25 BRITISH MEDICAL RESEARCH COUNCIL'S COMMITTEE ON THE AETIOLOGY OF CHRONIC BRONCHITIS Chronic bronchitis in Great Britain Brit med J II 973 1961

26 BRITISH MEDICAL RESEARCH COUNCIL'S COMMITTEE ON THE AETIOLOGY OF CHRONIC BRONCHITIS Definition and classification of chronic bronchitis For clinical and epidemiological purposes Lancet I 775 1965

27 BRITTINGHAM H H & WHITE P D Cardiac functional tests J Amer med Ass *79* 1901 1922

28 BROWN C C Jr FRY D L & EBERT R V Mechanics of pulmonary ventilation in patients with heart disease Amer J Med *17* 438 1954

29 BROWN F S Lung area from surface tension effects Proc Soc exp Biol *95* 168 1957

30 BRUNES L & HOLMGREN A Total airway resistance and its relationship to body size and lung volumes in healthy young women Scand J clin Lab Invest *18* 316 1966

31 BRUNS O Die Bedeutung der spirometrischen Untersuchung von Emphysematikern und Herzkranken Med Klin *6* 1524 1910

32 BUTLER J WHITE, H C & ARNOTT W M The pulmonary compliance in normal subjects Clin Sci *16* 709 1957

33 BUHLMANN A BEHN H & SCHUPPLI M Die Lungenfunktion bei chronischer Lungenstauung unter besonderer Berucksichtigung der Atemmechanik Schweiz med Wschr *89* 37 1959

34 BURGER, H C The significance of the flow volume diagram in the study of the mechanics of breathing Proc Tuberc Res Coun *46* 28 1959

35 BUYTENDIJK H J Oesophagusdruck en longelasticiteit Diss Groningen 1949

36 CHENG T O GODFREY M P & SHEPARD R H Pulmonary resistance and state of inflation of lungs in normal subjects and in patients with airway obstruction J appl Physiol *14* 727 1959

37 CHERNIACK R M The physical properties of the lung in chronic obstructive pulmonary emphysema J clin Invest *35* 394 1956

38 CHERNIACK R M CUDDY T E & ARMSTRONG J B Significance of pulmonary elastic and viscous resistance in orthopnea Circulation *15* 859 1957

39 CHRISTIE R V & MEAKINS J C Intrapleural pressure in congestive heart failure and its clinical significance J clin Invest *13* 323 1934

40 CLOWES G H A Jr HACKEL, D B MUELLER R P & GILLESPIE D G Relationship of pulmonary functional and pathological changes in mitral stenosis Arch Surg *67* 244 1953

41 COHN J E & DONOSO H D Mechanical properties of lung in normal men over 60 years old. J clin Invest *42* 1406 1963

42 COOK, C D & HAMANN J A Relation of vital capacity to height in healthy persons between the age of 5 and 38 years J Pediat *59* 710 1961

43 COOK C D HELLIESEN P J & AGATHON, S Relation between mechanics of respiration lung size and body size from birth to young adulthood. J appl Physiol. *13* 349 1958

44 COOK C D MEAD J SCHREINER, G L FRANK, N R & CRAIG J M. Pulmonary mechanics during induced pulmonary edema in anesthetized dogs J appl Physiol *14* 177 1959

45 COTES J E ROSSITER, C E HIGGINS I T T & GILSON J C Average normal values for the forced expiratory volume in white Caucasian males Brit med J I 1016 1966

46 CRAIG A. B Jr Effects of position on expiratory reserve volume of the lungs J appl. Physiol *15* 59 1960

47 CURTI P C COHEN G CASTLEMAN B SCANNELL, J G FRIEDLICH A L. & MYERS G S Respiratory and circulatory studies of patients with mitral stenosis. Circulation *8* 893 1953

48 DAHLBERG G Statistical methods for medical and biological students George Allen & Unwin London 1948

49 DALY W D ROSS J C & BEHNKE R H The effect of changes in pulmonary vascular bed produced by atropine pulmonary engorgement and positive pressure breathing on diffusing and mechanical properties of the lung J clin Invest *42* 1083 1963

50 DAVIDSON J T WASSERMAN K. LILLINGTON G A. & SCHMIDT R W Effect of aging on respiratory mechanics and gas exchange in rabbits. J appl. Physiol. *21* 837 1966

51 DAYMAN H Mechanics of breathing In Pulmonary Emphysema Ed Barach A & Bickerman H, p 2?1 The Williams and Wilkins Company, Baltimore 1956

52 DEFARES J G & DONLEBEN P H Relationship between frequency dependent compliance and unequal ventilation J appl Physiol *15* 166 1960

53 DE GRAFF A C DE LA CHAPELLE C E EGGLESTON C KOSSMANN CH E LEWY R L PARDEE H E B & SCHWEDEL J B Nomenclature and criteria for diagnosis of diseases of the heart p 71 New York Heart Association New York 1942

54 DUBOIS A B BOTHELHO S Y & COMROE J H A new method for measuring airway resistance in man using a body plethysmograph values in normal subjects and in patients with respiratory disease J clin. Invest *35* 327 1956

55 DUBOIS, D & DUBOIS E F A formula to estimate the approximate surface area if height and weight be known Arch intern Med *17* 863 1916

56 EHRNER L Lung compliance and respiratory resistance Acta med scand Suppl 353 1960

57 EHRNER L & NISELL, O Variability of some characteristics of respiratory mechanics in normal adults Acta med scand *164* 95 1959

58 ENGSTROM I KARLBERG P & KRAEPELIN, S Respiratory studies in children. I Lung volumes in healthy children 6–14 years of age Acta paediat (Uppsala) *45* 277 1956

59 ESPERSEN T Congestive heart failure oxygen consumption and lung ventilation Acta med scand *108* 183 1941

60 FENN W O & RAHN H editors Handbook of Physiology Sect 3 Respiration I–II American Physiological Society Washington 1964–1965

61 FERRIS B G Jr & ANDERSON D O The prevalence of chronic respiratory disease in a New Hampshire town Amer Rev resp Dis 86 165 1962

62 FERRIS B G Jr ANDERSON D O & ZICKMANTEL R Prediction values for screening tests of pulmonary function Amer Rev resp Dis 91 252 1965

63 FERRIS B G Jr MEAD J & OPIE L H. Partitioning of respiratory flow resistance in man J appl Physiol. 19 653 1964

64 FERRIS B G Jr & POLLARD D S Effect of deep and quiet breathing on pulmonary compliance in man J clin Invest 39 143 1960

65 FLETCHER, C M ELMES P C FAIRBAIRN A S & WOOD C H The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population Brit med J 2 257 1959

66 FORSBERG S A Pulmonary blood volume in man Acta med scand Suppl 410 1964

67 FRANK N R Influence of acute pulmonary vascular congestion on recoiling force of excised cats lung J appl Physiol 14 905 1959

68 FRANK N R CUGELL D W GAENSLER, A E & ELLIS L B Ventilatory studies in mitral stenosis A comparison with findings in primary pulmonary disease Amer J Med. 15 60 1953

69 FRANK N R LYONS H A SIEBENS A A & NEALON T F Pulmonary compliance in patients with cardiac disease Amer J Med, 22 516 1957

70 FRANK N R MEAD J & FERRIS B G Jr The mechanical behavior of the lungs in healthy elderly persons. J clin Invest 36 1680 1957

71 FRANK N R MEAD J SIEBENS A A & STOREY C F Measurements of pulmonary compliance in seventy healthy young adults. J appl Physiol 9 38 1956

72 FRIEDMAN B L MACIAS J & YU P N Pulmonary function studies in patients with mitral stenosis Amer Rev Tuberc 79 265 1959

73 FRY D L Theoretical considerations of the bronchial pressure flow volume relationships with particular reference to the maximum expiratory flow volume curve Phys in Med Biol 3 174 1958

74 FRY D L EBERT R V STEAD W W & BROWN C C The mechanics of pulmonary ventilation in normal subjects and in patients with emphysema Amer J Med 16 80 1954.

75 FRY D L & HYATT R E Pulmonary mechanics A unified analysis of the relationship between pressure volume and gasflow in the lungs of normal and diseased human subjects Amer J Med. 29 672 1960

76 FRY D L STEAD W W EBERT R V, LUBIN R L & WELLS H S The measurement of intraesophageal pressure and its relationship to intrathoracic pressure J Lab clin Med 40 664 1952

77 GAENSLER, E A. Analysis of ventilatory defect by timed capacity measurements Amer Rev Tuberc 64 256 1951

78 GARDAM J D Vital capacity in adults with heart disease Its relation to age degree of cardiac enlargement and type of valvular lesion Amer J med Sci 219 76 1950

79 GAZETOPOULOS N DAVIES H OLIVER C & DEUCHAR D Ventilation and hemodynamics in heart disease Brit Heart J 28 1 1966

80 GILSON J C & HUGH JONES P The measurement of the total lung volume and breathing capacity Clin Sci 7 185 1949

81 GIUNTINI C MASERI A & BIANCHI R Pulmonary vascular distensibility and lung compliance as modified by Dextran infusion and subsequent atropine injection in normal subjects J clin Invest 45 1770 1966

82 GOLDMAN, H J & BECKLAKE M R Respiratory function tests normal values at median altitudes and the prediction of normal results Amer Rev Tuberc *79* 457 1959

83 GOLDSMITH J R A simple test of maximal expiratory flow for detecting ventilatory obstruction Amer Rev Tuberc *78* 180 1958

84 GOUGH J Correlation of radiological and pathological changes in some diseases of the lung Lancet I *161* 1955

85 GOUGH J The lungs in mitral stenosis In Recent advances in Pathology Ed *Harrison C V p 57 J & A.* Churchill Ltd London 1960

86 GRANATH A Mitral valvulotomy A clinical and hemodynamic pre and postoperative study Acta med scand 178 Suppl. 433, 1965

87 GRIMBY, G & SÖDERHOLM, B Spirometric studies in normal subjects III Static lung volumes and maximum voluntary ventilation in adults with a note on physical fitness Acta med scand *173* 199, 1963

88 HANSON J S TABAKIN, B S & CALDWELL, E J Response of lung volumes and ventilation to posture change and upright exercise J appl Physiol *17* 783 1962

89 HARRIS P & HEATH D The human pulmonary circulation Its form and function in health and disease E & S Livingstone Edinburgh 1962

90 HARRISON T R, TURLEY, F C, JONES, E & CALHOUN J A Congestive heart failure X. The measurement of ventilation as a test of cardiac function Arch. intern Med *48* 377 1931

91 HAYEK H *Die anatomischen Grundlagen des Lungenodems* Wien med. Wschr *65* 740 1953

92 HAYWARD G W & KNOTT J M S The effect of exercise on lung distensibility and respiratory work in mitral stenosis Brit Heart J *17* 303 1955

93 HEATH D & HICKEN, P The relation between left atrial hypertension and lymphatic distension in lung biopsies Thorax *15* *54* 1960

94 HELLIESEN P J COOK, C D FRIEDLANDER, L & AGATHON, S Studies of respiratory physiology in children I Mechanics of respiration and lung volume in 85 normal children 5 to 17 years of age Pediatrics *22* 80, 1958

95 HEWLETT, A W The vital capacities of patients with cardiac complaints Heart *11* 195 1924

96 HEYER H E *HOLMAN J & SHIRES G T* The diminished efficiency and altered dynamics of respiration in experimental pulmonary congestion Amer Heart J *35* 463 1948

97 HIGGINS I T T Respiratory symptoms bronchitis, and ventilatory capacity in random sample of an agricultural population Brit med J II *1198* *1957*

98 HIGGINS I T T & COCHRAN J B Respiratory symptoms bronchitis and disability in a random sample of an agricultural community in *Dumfries*shire Tubercle *39* 296 1958

99 HOLMGREN A Determination of the functional residual volume by means of the helium dilution method Scand J clin Lab Invest *6* *131* *1954*

100 HOWELL J B L PERMUTT S PROCTOR D F, & RILEY, R L Effect of inflation of the lung on different parts of pulmonary vascular bed J appl Physiol *16* 71 1961

101 HUGHES R, MAY A J, & WIDDICOMBE, J C The effect of pulmonary congestion and edema on lung compliance J Physiol (London) *142* 306 1958

102 HUTCHINSON J On the capacity of the lungs and on the respiratory functions with a view of establishing precise and easy method of detecting disease by the spirometer Trans med Soc Lond *29* 137 *1846*

103 HYATT R E, Dynamic lung volumes. In Handbook of Physiology Sect 3 Respiration II p 1381 American Physiological Society Washington 1965

104 HYATT R E SCHILDER, D P & FRY D L Relationship between maximum expiratory flow and degree of lung inflation J appl. Physiol 13 331 1958

105 INGRAM R H & SCHILDER D P Effect of thoracic gas compression on the flow volume curve of the forced vital capacity Amer Rev resp Dis 94 56 1965

106 JAEGER, M J & OTIS A B Effects of compressibility of alveolar gas on dynamics of breathing J appl Physiol. 19 83 1964a

107 JAEGER, M J & OTIS A B Measurement of airway resistance with a volume displacement body plethysmograph J appl. Physiol 19 813 1964b

108 JOHNSON R J Jr MILLER W F & WU N Timed forced expiratory volumes and pulmonary conductance Amer Rev resp Dis. 86 2-8 1962

109 JONSELL, S A A method for the determination of the heart size by teleroentgenography (a heart volume index) Acta radiol. (Stockh.) 20 325 1939

110 JORDAN S C HICKEN P WATSON D A., HEATH D & WHITAKER, W Pathology of the lungs in mitral stenosis in relation to respiratory function and pulmonary hemodynamics. Brit Heart J 28 101 1966

111 JULIN A & WILHELMSEN L Bronchial asthma and chronic bronchitis in a random population sample Scand J Resp Dis 48 330 1967

112 KJELLBERG S R. LONROTH H. & RUDHE, U The effect of various factors on the roentgenological determination of the cardiac volume. Acta radiol. (Stockh.) 35 413 1951

113 KJELLBERG S R., RUDHE U & SJOSTRAND T The relation of the cardiac volume to the weight and surface area of the body the blood volume and the physical capacity for work Acta radiol (Stockh) 31 113 1949

114 KOHN R R Changes in connective tissue in aging of the lung perspectives. Arch. environm. Hlth. 9 255 1964

115 KORSGREN M Personal communication 1967

116 KORSGREN M WILHELMSEN L BJURE J., & VARNAUSKAS E Pulmonary circulation diffusing capacity and mechanics of breathing in pulmonary arterial hypertension To be published

117 KORY R. C., CALLAHAN P., BOREN H. G & SYNER, J C The Veterans Administration—army cooperative study of pulmonary function I. Clinical spirometry in normal men. Amer J Med 30 243 1961

118 LARSSON H. & KJELLBERG S R Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol. (Stockh) 29 159 1948

119 LEATH D E & MEAD J Mechanisms determining residual volume of the lungs in normal subjects. J Appl. Physiol. 23 221 1967

120 LEUALLEN, E. C & FOWLER, W S Maximal mid-expiratory flow Amer Rev Tuberc 72 783 1955

121 LEVINE R MELLINS R B & FISHMAN A. P Quantitative assessment of pulmonary edema. Circulat Res 17 414 1965

122 LOCKHART W SMITH D H. MAIR, A., & WILSON W A. Practical experience with the peak flow meter Brit med J I. 37 1960

123 LUNDSGAARD CH. Determination and interpretation of changes in lung volumes in certain heart lesions. J Amer med. Ass. 80 163 1923

124 MacIntosh D J Sinnott J C Milne, I G, & Reid E A B Some aspects of disordered pulmonary function in mitral stenosis Ann intern Med 49 1294 1958

125 Mack I Grossman M & Katz L N The effect of pulmonary congestion on distensibility of the lung Fed Proc 6 161 1947

126 Marshall, R The physical properties of the lungs in relation to the subdivisions of lung volume Clin Sci 16 507 1957

127 Marshall, R Objective tests of respiratory mechanics In Handbook of Physiology Sect 3 Respiration II p 1399 American Physiological Society Washington 1965

128 Marshall R & DuBois A B The measurement of the viscous resistance of the lung tissues in normal man Clin Sci 15 161 1956

129 Marshall, R McIlroy M B & Christie R V The work of breathing in mitral stenosis Clin Sci 13 137 1954

130 Marshall R Stone R W & Christie R V The relationship of dyspnoea to respiratory effort in normal subjects mitral stenosis and emphysema Clin Sci 13 625 1954

131 McCall C B Hyatt R E Noble F W & Fry D L Harmonic content of certain respiratory flow phenomena of normal individuals J appl Physiol 10 215 1957

132 McCredie M Measurement of pulmonary edema in valvular heart disease Circulation 36 381 1967

133 McIlroy M B & Christie R V The work of breathing in emphysema. Clin Sci 13 147 1954

134 McIlroy M B Mead J Selverstone N J & Radford E P Measurement of lung tissue viscous resistance using gases of equal kinematic viscosity J appl. Physiol 7 485 1955

135 McIlroy M B Tierney D F, & Nadel, J A A new method for measurement of compliance and resistance of lungs and thorax J appl Physiol 18 424, 1963

136 Mead J Mechanical properties of lungs Physiol Rev 41 281 1961

137 Mead J & Collier C Relation of volume history of lungs to respiratory mechanics in anesthetized dogs J appl Physiol 14 669 1959

138 Mead J Frank N R Lindgren, I Gaensler E A & Whittenberger J L A technic for the measurement of pulmonary compliance and resistance Its application to normal patients and patients with mitral stenosis Clin Res Proc 1 116 1953

139 Mead J & Gaensler E A Esophageal and pleural pressures in man upright and supine J appl Physiol 14 81 1959

140 Mead J Lindgren I & Gaensler E A Mechanical properties of the lungs in emphysema J clin Invest 34 1005 1955

141 Mead J Turner J M, Macklem P T & Little J B Significance of the relationship between lung recoil and maximum expiratory flow J appl Physiol 22 95 1967

142 Mead J & Whittenberger J L Physical properties of human lungs measured during spontaneous respiration J appl Physiol 5 779 1953

143 Meakins J C & Christie R V Lung volume and its variations Ann intern Med 3 423 1929

144 Meneely G R & Kaltreider N L The volume of the lung determined by helium dilution description of the method and comparison with other procedures J clin Invest 28 129 1949

145 Milic Emili J Mead J Turner J M & Glauser E M Improved technique for estimating pleural pressure from esophageal balloons J appl Physiol 19 207 1964a

146 MILIC EMILI J MEAD J & TURNER J M Topography of esophageal pressure as a function of posture in man J appl Physiol *19* 212 1964*b*

147 MILLER, W F JOHNSON R L Jr & WU N Relationships between fast vital capacity and various timed expiratory capacities J appl. Physiol *14* 157 1959

148 MILLS R J, CUMMING G & HARRIS P Frequency dependent compliance at different levels of inspiration in normal adults J appl Physiol *18* 1061 1963

149 MITTMAN C EDELMAN N H NORRIS A H & SHOCK N W Relationship between chest wall and pulmonary compliance and age J appl Physiol *20* 1211 1965

150 MORENO F & LYONS H A Effect of body posture on lung volumes J appl Physiol *16* 27 1961

151 MORSE M SCHULTZ F W & CASSELS D E The lung volume and its subdivisions in normal boys 10–17 years of age J clin Invest *31* 380 1952

152 MOUNT L E Ventilation flow resistance and compliance of rat lungs J Physiol (Lond) 127 157 1955

153 NADEL J A & COMROE J H Jr Acute effects of inhalation of cigarette smoke on airway conductance J appl. Physiol. *16* 713 1961

154 NADEL, J A. & TIERNEY, D F Effect of a previous deep inspiration on airway resistance in man. J appl Physiol. *16* 717 1961

155 NEEDHAM C D ROGAN M C & McDONALD I Normal standards for lung volumes intrapulmonary gas mixing and maximum breathing capacity Thorax *9* 313 1954

156 NISELL, O CARLBERGER G & BEVEGÅRD S The mechanics of respiration in patients with mitral heart disease Acta med. scand. *162* 277 1958

157 OSWALD N C & MEDVEI V C Chronic bronchitis The effect of cigarette smoking Lancet II 843 1955

158 OTIS A B McKERROW C B BARTLETT R A MEAD J McILROY M B SELVERSTONE N J & RADFORD E P Jr Mechanical factors in distribution of pulmonary ventilation J appl Physiol *8* 427 1956

159 PALMER W H GEE J B L MILLS F C & BATES D V Disturbances of pulmonary function in mitral valve disease Canad med Ass J *89* 744, 1963

160 PARKER F P & WEISS S Nature and significance of structural changes in lungs in mitral stenosis Amer J Pathol *12* 573 1936

161 PAULIN S & VARNAUSKAS E Selective transseptal angiocardiography Acta radiol. (Stockh) *57* 3 1962

162 PEABODY F W Clinical studies on the respiration III. A mechanical factor in the production of dyspnea in patients with cardiac disease Arch. intern Med *20* 433 1917

163 PEABODY F W & WENTWORTH J A Clinical studies of the respiration. IV The vital capacity of the lungs and its relation to dyspnea Arch intern Med *20* 443 1917

164 PEMBERTON J & FLANAGAN E G Vital capacity and timed vital capacity in normal men over 40 J appl Physiol *9* 291 1956

165 PERMUTT S BROMBERGER BARNEA B & BANE H N Alveolar pressure pulmonary venous pressure and the vascular waterfall. Medicina Thoracalis *191* 239 1962

166 PERMUTT S & MARTIN H B Static pressure volume characteristics of lungs in normal males J appl Physiol. *15* 819 1960

167 PETERS J P Jr & BARR D P Studies of the respiratory mechanism in cardiac dyspnea. II. A note on the effective lung volume in cardiac dyspnea. Amer J Physiol *54* 335 1920

168 PETIT J M SENTERRE J BOCCAR, M DELREZ M, & DAMOISEAU J Variabilite de la compliance pulmonaire pendant la ventilation de repos chez l adulte normal Path et Biol *10* 1179 1962

169 PIERCE J A & EBERT P V The elastic properties of the lungs in the aged J Lab clin Med *51* 63 1958

170 PIERCE, J A & HOCOTT J B Studies on the collagen and elastin content of the human lung J clin Invest *39* 8 1960

171 PRYOR W N HICKAM J B SIEKER H O & PAGE E B Effect of circulatory changes on the pulmonary compliance of normal subjects and patients with mitral stenosis Circulation *15* 721 1957

172 RAHN H OTIS A B CHADWICK L E, & FENN W O The pressure volume diagram of the thorax and lung Amer J Physiol *146* 161 1946

173 RAU G VON BEHN H GEBHARDT W ROSSIER P H & BUHLMANN A Atemmechanische Untersuchungen am Lungenmodell bei Lungengesunden und bei Patienten mit obstruktivem Emphysem Schweiz med Wschr *87* 374 1957

174 RICHARDS D G B WHITFIELD A G W, ARNOTT W M & WATERHOUSE J A H The lung volume in low output cardiac syndromes Brit Heart J *13* 381 1951

175 RINGQVIST T The ventilatory capacity in healthy subjects An analysis of causal factors with special reference to the respiratory forces Scand J clin Lab Invest Suppl 88 1966

176 ROBERTSSON B In Conference on pulmonary circulation Ed Muller C p 186 Universitetsforlaget Oslo 1965

177 SAID S I AVERY M E DAVIS R K, BANERJEE C M & EL GOHARY M Pulmonary surface activity in induced pulmonary edema J clin Invest *44* 458 1965

178 SAXTON G A JR, RABINOWITZ M, DEXTER L & HAYNES F The relationship of pulmonary compliance to pulmonary vascular pressures in patients with heart disease J clin Invest *35* 611, 1956

179 SCHILDER D P, ROBERTS A FRY D L Effect of gas density and viscosity on the maximal expiratory flow volume relationship J clin Invest *42* 1705 1963

180 SCHMIDT A M & COHN J E Modified body plethysmograph for study of cardiopulmonary physiology J appl Physiol *16* 935 1961

181 SCHULZ H Some remarks on the sub microscopic anatomy and pathology of the blood air pathway in the lung In Ciba Foundation Symposium on Pulmonary Structure and Function, p 209 J & A Churchill London 1962

182 SELBY T & READ, J Maximal expiratory flow rates in Australian adults Aust Ann Med *10* 49 1961

183 SERNIKAR I & MESCHIA G Proprièta elastische del polmone e di modelli meccanici Arch Fisiol *52* 288 1952

184 SHARP J T BUNNELL I L GRIFFITH G T & GREENE D G The effects of therapy on pulmonary mechanics in human pulmonary edema J clin Invest *40* 665 1961

185 SHARP J T GRIFFITH G T BUNNELL I L & GREENE D G Ventilatory mechanics in pulmonary edema in man J clin Invest *37* 111 1958

186 SIEBECK R Über die Beeinflussung der Atemmechanik durch krankhafte Zustande des Respirations und Kreislaufapparates Dtsch Arch klin Med *100* 204 1910

187 SNEDECOR G W Statistical Methods Ed 5 The Iowa State University Press Ames Iowa 1965

188 SOBOL B J & EMIRGIL C Subject effort and the expiratory flow rate Amer Rev resp Dis *89* 402 1964

189 STEAD, W W FRY D L & EBERT R V The elastic properties of the lung in normal men and in patients with chronic pulmonary emphysema J Lab clin Med. *40* 674 1952

190 STOCK J P P & KENNEDY M C S Ventilatory cost of exercise before and after mitral valvotomy Thorax *14* 238 1959

191 TIBBLIN G & WILHELMSEN L Results from the "Men of 1913 study" Chronic bronchitis in an urban population Lakartidn. *64* 2455 1967

192 TIERNEY D F & NADEL, J A Concurrent measurements of functional residual capacity by three methods J appl Physiol *17* 871 1962

193 TINKER C M Peak expiratory flow measured by the Wright peak flow meter Distribution of values in men aged 20–59 who denied respiratory symptoms Brit med. J I 1365 1961

194 TURINO G M Pulmonary distensibility in mitral stenosis and congenital heart disease J clin Invest *35* 740 1956

195 TURINO G M & FISHMAN A P The congested lung J chron Dis *9* 510 1959

196 TURNER J M The effect of age on the elastic properties of the lung the maximum expiratory flow volume curve and their interrelationship Fed Proc *22* 280 1963

197 VARNAUSKAS E & KORSGREN M The effect of plasma expansion by Rheomacrodex® on pulmonary blood volume and pulmonary water space Paper presented at the meeting in European Society for Clinical Investigation Noordwijk, Holland, April 28–29 1967

198 VERSTRAETEN J M VAN DER STRAETEN M VERSTRAETEN J & PANNIER R L elasticite pulmonaire au cours de la stenose mitrale Acta cardiol (Brux) *13* 262 1958

199 WEST J B DOLLERY, C T & HEARD, B E Increased pulmonary vascular resistance in the dependent zone of the isolated dog lung caused by perivascular edema. Circulat Res *17* 191 1965

200 WEST J R BLISS H A WOOD J A & RICHARDS D W Jr Pulmonary function in rheumatic heart disease and its relation to exertional dyspnea in ambulatory patients Circulation *8* 178 1953

201 WHITE H C BUTLER, J & DONALD K W Lung compliance in patients with mitral stenosis Clin Sci *17* 667 1958

202 WILHELMSEN L Pulmonary mechanics and pulmonary circulation in heart disease Conference on pulmonary circulation p 169 Universitetsforlaget Oslo 1965

203 WILHELMSEN L An investigation of handicapped persons in Goteborg Lakartidn. *64* 800 1967

204 WILHELMSEN L & TIBBLIN G Tobacco smoking in fifty year old men I Respiratory symptoms and ventilatory function tests Scand J Resp Dis *47* 121 1966

205 WILHELMSEN L TIBBLIN, G & ELD P The ventilatory capacity in a random sample of 50 year old men To be published

206 WILHELMSEN L & VARNAUSKAS E Effects of acute plasma expansion on the mechanics of breathing Clin. Sci *33* 29 1967

207 WOOD E ANTHONISEN N R & MACKLEM P T Personal Communication 1967

208 WRIGHT B M. & MCKERROW C B Maximum forced expiratory flow rate as a measure of ventilatory capacity Brit Med J II 1041 1959

209 WRIGHT R R Elastic tissue of normal and emphysematous lungs Amer J Path. *39* 355 1961

168 Petit J M, Sentebre, J Boccar M Delree M, & Damoiseau J Variabilité de la compliance pulmonaire pendant la ventilation de repos chez l'adulte normal Path et Biol *10* 1179 1962

169 Pierce J A & Ebert R V The elastic properties of the lungs in the aged J Lab clin Med *51* 63 1958

170 Pierce J A & Hocott J B Studies on the collagen and elastin content of the human lung J clin Invest *39* 8 1960

171 Prior W A, Hickam J B, Sieker H O & Page E B Effect of circulatory changes on the pulmonary compliance of normal subjects and patients with mitral stenosis Circulation *15* 721 1957

172 Rahn H Otis A B Chadwick L E & Fenn W O The pressure volume diagram of the thorax and lung Amer J Physiol *146* 161 1946

173 Rau G von Blan H Gebhardt W Rossier P H & Buhlmann A Atemmechanische Untersuchungen am Lungenmodell bei Lungengesunden und bei Patienten mit obstruktivem Emphysem Schweiz med Wschr *87* 374 1957

174 Richards D G B Whitfield A C W Arnott W M & Waterhouse J A H The lung volume in low output cardiac syndromes Brit Heart J *13* 381 1951

175 Rivqqvist T The ventilatory capacity in healthy subjects An analysis of causal factors with special reference to the respiratory forces Scand J clin Lab Invest Suppl 88 1966

176 Robertsson B In Conference on pulmonary circulation Ed Muller C p 186 Universitetsforlaget Oslo 1965

177 Said S I Avery M E Davis R K Banerjee C M & El Gohary M Pulmonary surface activity in induced pulmonary edema J clin Invest *44* 458 1965

178 Saxton G A Jr, Rabinowitz M, Dexter L, & Haynes F The relationship of pulmonary compliance to pulmonary vascular pressures in patients with heart disease J clin Invest *35* 611, 1956

179 Schilder D P Roberts A Fry D L Effect of gas density and viscosity on the maximal expiratory flow volume relationship J clin Invest *42* 1705, 1963

180 Schmidt A M & Cohn J E Modified body plethysmograph for study of cardiopulmonary physiology J appl Physiol *16* 935 1961,

181 Schulz H Some remarks on the submicroscopic anatomy and pathology of the blood air pathway in the lung In Ciba Foundation Symposium on Pulmonary Structure and Function, p 205 J & A Churchill London 1962

182 Selby T & Read J Maximal expiratory flow rates in Australian adults Aust Ann Med *10* 49 1961

183 Servaar I & Meschia G Proprietà elastiche del polmone e di modelli meccanici Arch Fisiol *52* 288 1952

184 Sharp J T Bunnell I L Griffith, G T & Greene D G The effects of therapy on pulmonary mechanics in human pulmonary edema J clin Invest *40* 665 1961

185 Sharp J T Griffith G T, Bunnell I I & Greene D G Ventilatory mechanics in pulmonary edema in man J clin Invest *37* 111 1958

186 Siebeck R Über die Beeinflussung der Atemmechanik durch krankhafte Zustande des Respirations und Kreislaufapparates Dtsch Arch klin Med, *100* 204 1910

187 Snedecor G W Statistical Methods Ed 5 The Iowa State University Press Ames Iowa 1965

188 Sobol B J & Emirgil C Subject effort and the expiratory flow rate Amer Rev resp Dis *89* 402 1964

APPENDIX

Table 1 Clinical findings in the different series of patients

Main diagnosis	No	Functional class N.Y.H.A. I	II	III	IV	Rhythm SR	AF	Cough and sputum: Only some-times	3 m a year	Wheezing: Some times	Most days	Sympt depending on weather	Dyspnoea grade 1	2	3	4	Tobacco smoking g/day 0	x*	1-14	15-24	≥25
Series (c)																					
Mitral stenosis	33	5	18	9	1	17	16	4	5	9	5	7	12	16	3	2	18	5	9	—	—
Mitral insuff (+aortic valv dis in one case)	5	1	2	2	—	1	4	1	1	2	1	2	3	—	2	—	4	1	—	—	—
Combined mitral stenosis and aortic valv dis	8	2	3	2	1	4	4	3	2	2	3	—	3	2	—	3	3	1	3		1
Aortic valv dis	5	3	2	—	—	5	—	2	—	1	—	1	4	1	—	—	4	—	1		—
Series (d)																					
Mitral stenosis (slight insuff in 1 case)	10	—	3	5	2	4	6	5	3	6	3	1	1	2	5	2	6	—	4		—
Series (e 1)																					
Mitral stenosis in 6 cases Mitral insuff and syst hypert in 1 case	7	—	4	3	—	2	5	3	1	4	1	1	1	3	3	—	4	1	2		—
Series (e 2)																					
3 cases who also included series (d) are excluded	4	—	3	1	—	1	3	1	0	2	1	1	1	2	1	—	2	1	1		
Total (c+d+e 2)	65	11	31	19	4	32	33	16	11	22	13	12	24	23	11	7	37	9	18		

* = ex smokers

Table 2 Control subjects (33 men and 39 women) Mean values ($\bar{x}$) and standard errors ($s_{\bar{x}}$) and ranges for lung mechanics

	Males		(n=33)	Females		(n=39)
	$\bar{x}$	$s_{\bar{x}}$	range	$\bar{x}$	$s_{\bar{x}}$	range
Age years	42 [illegible]	2 [illegible]	18– 70	43 0	2 [illegible]	19– 73
Height cm	177	1 2	160–192	164	0 96	152–179
Weight kg	75 2	1 [illegible]	57–102	64 0	1 8	45– 99
TLC L	7 10	0 18	5 32 – 9 51	5 06	0 13	3 34 – 7 03
RV, L	1 85	0 10	0 91 – 3 10	1 48	0 06	0 83 – 2 9
FRC L	3 68	0 15	2 30 – 5 51	2 64	0 10	1 52 – 4 27
VC L	5 25	0 14	3 48 – 7 11	3 [illegible]8	0 11	2 06 – 5 12
FEV₁ L	4 04	0 11	2 66 – 5 79	2 83	0 10	1 41 – 4 0[illegible]
FEV₁ %	77 3	1 0	68 6 – 89 2	78 4	1 0	60 [illegible] – 90 6
PEF LPS	8 1	0 3[illegible]	4 83 – 13 15	5 66	0 29	2 5[illegible] – 10 90
MEF50% LPS	5 01	0 26	3 1[illegible] – 9 36	3 80	0 19	1 29 – 6 8[illegible]
MEF25% LPS	1 [illegible]9	0 12	0 69 – 3 3[illegible]	1 66	0 12	0 49 – 4 2[illegible]
4(*MEF50%–MEF25%*)/*VC* 1/sec	2 49	0 15	1 41 – 6 16	2 3[illegible]	0 16	0 [illegible]0 – 5 02
4 *MEF25%/VC* 1/sec	1 3[illegible]	0 09	0 55 – 2 [illegible]6	1 84	0 11	0 89 – 3 86
MEF50%–MEF25%/MEF25%	2 01	0 14	0 91 – 4 30	1 31	0 13	0 28 – 3 9[illegible]
Cdyn(l) L/cm H_2O	0 229	0 013	0 132– 0 402	0 155	0 006	0 096– 0 232
f breaths/min	17 6	0 [illegible]1	11 –25	18 3	0 9	6 – 31
Ri(l) cm H_2O/*LPS*	1 10	0 0[illegible]	0 [illegible] – 2 38	1 65	0 08	0 93 – 3 33
Gi(l) LPS/cm H_2O	1 02	0 06	0 42 – 1 82	0 65	0 03	0 30 – 1 08
Ri(l) Cdyn(l) sec	0 2[illegible]6	0 0[illegible]	0 106 – 0 74[illegible]	0 248	0 011	0 12 – 0 407

Table [illegible] Control subjects (33 men and 39 women) Multiple regression equations with age (A), height (H) in cm weight (W) in kg and lung volumes as independent variables Only significant ($p<$[illegible]) equations given Standard errors of regression coefficients in parentheses

Eq no	Sex	Dependent variable	Independent variables	Constant	RSD	R^2
1	M	TLC	$+0$ 08243 H (± 0 022[illegible]2)	-7 4 2	0 8 0	0 29
2	F	$TLC=$	$+0$ 0[illegible]739 H (± 0 01636)	-7 646	0 598	0 36
3	M	$RV=$	$+0$ 0[illegible]957 $A+0$ 3621 TLC (± 0 00380) (± 0 0575)	-1 603	0 332	0 69
4	F	$RV=$	$+0$ 012[illegible]9 $A+0$ [illegible]113 TLC (± 0 00320) (± 0 0 29)	-0 645	0 310	0 41
5	M	$FRC=$	$+0$ 06381 H (± 0 0[illegible]015)	$-$[illegible] 600	0 769	0 [illegible]4
6	F	$FRC=$	$+0$ 05[illegible]42 $H-0$ 01616 W (± 0 01463) (± 0 00[illegible]68)	-5 430	0 52[illegible]	0 31
7	M	$FRC=$	$+0$ 5[illegible]01 TLC (± 0 0946)	-1 0 8	0 [illegible]16	0 62
8	F	$FRC=$	$+0$ 6[illegible]59 FVC (± 0 0761)	-0 8[illegible]1	0 350	0 69
9	M	$VC=$	-0 01[illegible]26 $A+0$ 06656 H (± 0 00693) (± 0 01586)	$-$[illegible] 79	0 595	0 49
10	F	$VC=$	-0 01690 $A+0$ 049[illegible]8 H (± 0 00[illegible]39) (± 0 01548)	-3 834	0 [illegible]11	0 49
11	M	$VC=$	-0 0[illegible]05[illegible] $A+0$ 63[illegible]9 TLC (± 0 00380) (± 0 0[illegible]75)	$+1$ 603	0 332	0 64
12	F	$VC=$	-0 012[illegible]9 $A+0$ 668[illegible] TLC (± 0 00320) (± 0 0 29)	$+0$ 64[illegible]	0 310	0 81
13	M	$FEV_1=$	-0 02207 $A+0$ 038[illegible] H (± 0 00[illegible]34) (± 0 01221)	-1 813	0 453	0 52
14	F	$FEV_1=$	-0 01698 $A+0$ 03822 H (± 0 00491) (± 0 01402)	-2 [illegible]16	0 465	0 48
15	M	$FEV_1=$	-0 01140 $A+0$ 6086 VC (± 0 00348) (± 0 0648)	$+1$ 3[illegible]	0 268	0 64
16	F	$FEV_1=$	$+0$ 8[illegible]68 VC (± 0 0[illegible]25)	-0 1[illegible]4	0 231	0 87
17	M	$FEV_1\%=$	-0 1811 $A-0$ 9[illegible]9 H (± 0 0 91) (± 0 13[illegible])	$+136$	5 0 0	0 [illegible]9
19	M	$PEF=$	-0 0 542 $A+0$ 09696 H (± 0 01991) (± 0 05085)	$+4$ 48	1 [illegible]59	0 40
20	F	$PEF=$	$+1$ 0516 TLC (± 0 3815)	$+0$ 232	1 665	0 19
22	F	$PEF=$	$+1$ 4018 VC (± 0 3609)	$+0$ [illegible]36	1 [illegible]55	0 29
23	M	$MEF\,50\%$	-0 05041 A (± 0 01487)	$+7$ 158	1 81	0 28
24	F	$MEF\,50\%=$	-0 0[illegible]303 A (± 0 01086)	$+4$ [illegible]67	1 140	0 11

Eq.no	Sex	Dependent variable	Independent variables	Constant	PSD	P^2
26	F	MEF50%=	−0 6[illegible]32 TLC (±0 2412)	+0 642	1 111	0 1[illegible]
28	F	MEF50%=	−0 9 99 VC (±0 2346)	−0 467	1 011	0 30
29	M	MEF25%=	−0 02 44 A (±0 00659)	−2 93[illegible]	0 5[illegible]6	0 23
30	F	MEF25%=	−0 0[illegible]647 A (±0 00556)	−2 793	0 555	0 33
32	F	MEF25%=	+0 39[illegible]8 TLC (±0 1477)	−0 328	0 6[illegible]0	0 16
34	F	MEF25%=	+0 5306 VC (±0 1485)	−0 240	0 640	0 28
36	F	A(MEF50%−MEF25%)/VC=	+0 01944 A (±0 00917)	+1 3 07	0 982	0 11
37	M	A MEF25%/VC=	−0 01504 A (±0 00529)	+2 067	0 461	0 21
38	F	A MEF25%/VC=	−0 01412 A (±0 00545)	−2 575	0 614	0 19
39	M	MEF50%−MEF25%/MEF25%=	+0 01834 A (±0 008 3)	−1 229	0 782	0 13
40	F	MEF50%−MEF25%/MEF25%=	+0 02409 A (±0 00 05)	−0 4 1	0 739	0 24
41	M	Cdyn(l)=	−0 03249 TLC (±0 01133)	−0 0015	0 0654	0 21
42	F	Cdyn(l)=	−0 01868 TLC (±0 00 1)	−0 0601	0 0529	0 15
43	M	Cdyn(l)=	−0 00512 A+0 05108 VC (±0 000 8) (±0 01159)	−0 1621	0 0599	0 36
44	F	Cdyn(l)=	−0 00081 A+0 02482 VC (±0 00039) (±0 00949)	−0 0237	0 0327	0 19
46	F	Cdyn(l)=	+0 01861 FPC (±0 00885)	+0 1050	0 0338	0 11
48	F	Gt(l)=	−0 0900 TLC (±0 034)	+0 198	0 160	0 15
50	F	Gt(l)=	−0 052 FVC (±0 0430)	+0 417	0 164	0 11
51	M	Gt(l)=	−0 19 4 VC (±0 0 09)	−0 012	0 323	0 20
52	F	Gt(l)=	−0 0823 VC (±0 0380)	+0 559	[illegible] 164	0 11
53	M	Pt(l) Cdyn(l)=	+0 00264 A (±0 001 0)	−0 1005	[illegible] 1458	0 13

Table 4 Control subjects (20 women) studied for static recoil pressure—$Pst(l)$—at different percentages of TLC

$Pst(l)$ cm H_2O	Per cent of total lung capacity 30	40	50	60	70	80	90	100
$\bar{x}$	2.3	4.1	6.2	8.3	10.6	13.5	17.9	28.2
$s_{\bar{x}}$	0.5	0.5	0.5	0.6	0.6	0.6	0.8	1.2
Range	0.2–6.3	0.7–7.8	2.2–10.0	3.6–12.9	5.4–15.5	8.2–19.7	12.9–27.3	[illegible].6–41.2
n	11	18	20	20	20	20	20	20

Table 5 Control subjects (20 women) studied for static compliance—$Cst(l)$—at normal FRC. Multiple regression equations with age (A), height (H) and lung volumes as independent variables. Only significant ($p<0.0$[illegible]) equations given. Standard errors for regression coefficients given in parentheses

Dependent variable	Independent variable	Constant	RSD	R^2
$Cst(l)$ =	+0.00329 H (±0.001[illegible]1)	−0.3302	0.0486	0.21
$Cst(l)$ =	+0.04348 TLC (±0.00952)	−0.0071	0.0371	0.[illegible]3
$Cst(l)$ =	+0.0[illegible]148 FRC (±0.01822)	+0.0865	0.0454	0.31
$Cst(l)$ =	+0.002207 A+0.06[illegible]61 VC (±0.000630) (±0.01480)	−0.1154	0.0289	0.52
$Cst(l)$ =	+0.0337[illegible] VC (±0.011[illegible]2)	+0.0864	0.0449	0.32

Table 6 Control subjects (20 women) The effect of correcting flow volume curves for alveolar gas compression on the maximum expiratory flow values ($\dot{V}max$, LPS)

$\dot{V}max$ LPS	Per cent of vital capacity 10	20	30	40	50	60	70
Not corrected							
$\bar{x}$	0.41	1.04	2.01	3.02	4.04	4.90	5.[illegible]8
$s_{\bar{x}}$	0.05	0.11	0.[illegible]0	0.25	0.30	0.34	0.33
Range	0.16–0.94	0.42–2.09	0.[illegible]–4.09	1.06–5.43	2.11–6.85	2.4[illegible]–7.85	3.27–8.88
Corrected							
$\bar{x}$	0.59	1.43	2.46	3.51	4.56	5.39	6.16
$s_{\bar{x}}$	0.06	0.13	0.23	0.27	0.31	0.33	0.3[illegible]
Range	0.27–1.16	0.74–2.[illegible]	0.99–4.[illegible]6	1.81–6.10	2.51–7.15	3.18–8.42	4.0[illegible]–9.64
$\bar{d}$	0.18	0.39	0.45	0.49	0.52	0.49	0.38
Signif of diff $p<$	0.001	0.001	0.001	0.001	0.001	[illegible].001	0.001

Table 7 Control subjects (20 women) Peripheral airway or upstream conductance (*Gus* $LPS/cm\ H_2O$) at different percentages of *VC*

Gus $LPS/cm\ H_2O$	Per cent of vital capacity 10	20	30	40	50	60	70
$\bar{x}$	0 30	0 40	0 46	0 50	0 [illegible]3	0 52	0 49
$s_{\bar{x}}$	0 05	0 0[illegible]	0 06	0 05	0 0[illegible]	0 04	0 04
Range	0 0[illegible]–0 [illegible]5	0 11–1 [illegible]	0 14–1 06	0 21–1 13	0 [illegible]–1 05	0 2[illegible]–0 [illegible]	0 [illegible]9–0 94

Table 8 Control subjects ([illegible]0 women) Peripheral airway or upstream conductance (*Gus* $LPS/cm\ H_2O$) Simple linear regression equations with *Gus* at different percentages of *VC* as dependent and age (*A*) *TLC* and *VC* as independent variables. Only significant ($p<0\ 05$) equations given

Dependent variable	Independent variable	Constant	*RSD*	R^2
Gus at 10% *VC* =	−0 0084[illegible] *A* (±0 0033[illegible])	+0 [illegible]00	[illegible]233	0 [illegible]6
Gus at 20% *VC* =	−0 00914 *A* (±0 0040[illegible])	+0 81[illegible]	0 261	0 22
Gus at 40% *VC* =	−0 0081[illegible] *A* (±0 00304)	+0 671	0 210	0 29
Gus at 50% *VC* =	−0 00[illegible]38 *A* (±0 00[illegible]4)	+0 861	0 189	0 29
Gus at 50% *VC* =	+0 1161 *TLC* (±0 0[illegible]05)	−0 041	0 19[illegible]	0 23
Gus at 50% *VC* =	+0 1382 *VC* (±0 04[illegible]4)	+0 038	0 165	0 32
Gus at 60% *VC* =	−0 005[illegible] *A* (±0 00[illegible]49)	+0 [illegible]9	0 1[illegible]	0 23
Gus at 60% *VC*	+0 1186 *TLC* (±0 041[illegible])	−0 062	[illegible]163	0 31
Gus at 60% *VC* =	−0 1[illegible]94 *VC* (±0 0400)	+0 061	0 156	0 3[illegible]
Gus at 70% *VC*	+0 1122 *TLC* (±0 034[illegible])	−0 060	0 133	0 3[illegible]
Gus at 70% *VC* =	+0 1145 *VC* (±0 0338)	+0 034	0 132	0 39

Table ▮ 192 men aged 50 without respiratory symptoms. Regression equations of values for lung mechanics with height (cm), weight (kg), body surface area (m^2) and VC (L). Only significant ($p < 0.05$) equations given

Eq no	Dependent variable	Independent variable	Constant	*RSD*	R^2
1	$VC =$	$+0.058[illegible]9\ H$ (± 0.00699)	−5.354	0.588	0.64
2	$VC =$	$+0.6426\ H^2$ ($\pm 0.0[illegible]6$)	+1.4[illegible]9	0.588	0.63
3	$VC =$	$+0.0[illegible]209\ W$ (± 0.00520)	+3.2[illegible]9	0.658	0.54
4	$VC =$	$+2.4087\ BSA$ (± 0.3583)	+0.369	0.610	0.60
5	$FEV_1 =$	$+0.03989\ H$ (± 0.00580)	−3.203	0.468	0.20
6	$FEV_1 =$	$+0.4350\ H^2$ (± 0.063)	+1.439	0.489	0.20
7	$FEV_1 =$	$+0.6009\ VC$ (± 0.0374)	+0.812	0.355	0.58
8	$FEV_1\% =$	$+0.13337\ W - 3.410\ VC$ (± 0.05414) ($\pm 0.[illegible]22$)	+83.[illegible]4	6.54	0.11
9	$PEF_{(Wright)} =$	$+35.65\ VC$ ($\pm 6.6[illegible]$)	+346.1	63.3	0.13
10	$MEF50\% =$	$+0.5210\ VC$ (± 0.119)	+2.036	1.134	0.09

Height in meters in equations 2 and 6

Table 10 Men with rheumatic valvular disease Presence or absence of cough and sputum for three months a year related to values for lung mechanics

	Series without cough and sputum 3 months a year ($n=17$)				Series with cough and sputum 3 months a year ($n=3$)			
	Observed		Pred	Signif of difference	Observed		Pred	Signif of difference
	$\bar{x}$	$s_{\bar{x}}$	x*	$p<$	$\bar{x}$	$s_{\bar{x}}$	x*	$p<$
Age	45 0	2 5			52 3	3 2		
Height	173 9	1 2			176 0	1 2		
Weight	71 6	2 6			67 3	6 6		
TLC	5 60	0 24	6 86	0 001	7 16	0 70	7 04	—
RV	1 70	0 10	1 81	—	3 04	0 50	2 02	—
RV	1 70	0 10	1 3[illegible]	0 02	3 04	0 50	2 07	0 05
FRC	2 90	0 15	3 50	0 01	4 13	0 58	3 63	—
VC	3 90	0 [illegible]0	5 02	0 001	4 12	0 40	5 04	—
FEV$_1$	2 93	0 21	3 88	0 001	2 91	0 44	3 83	—
FEV$_1$%	75 9	2 2	77 7	—	68 7	3 3	75 6	0 05
PEF	6 23	0 38	8 18	0 001	6 [illegible]4	1 16	7 20	—
MEF 50%	3 51	0 27	4 89	0 001	2 60	0 68	4 52	0 02
MEF 25%	1 34	0 20	1 72	—	1 07	0 3[illegible]	1 52	—
Cdyn(l)	0 156	0 013	0 220	0 001	0 156	0 014	0 237	0 001
Cdyn(l)	0 156	0 013	0 156	—	0 156	0 014	0 185	0 05
f	11 3	1 3			16 7	4 7		
G$_1$*(l)*	0 57	0 05	0 98	0 001	0 [illegible]0	0 25	0 94	—
G$_1$*(l)*	0 57	0 05	0 76	0 02	0 [illegible]0	0 25	0 80	—

* Predicted value based on predicted *TLC* or *VC*

Table 11 Women with rheumatic valvular disease Presence or absence of cough and sputum for three months a year related to values for lung mechanics

	Series without cough and sputum 3 months a year ($n=26$)				Series with cough and sputum 3 months a year ($n=5$)			
	Observed		Pred	Signif of difference	Observed		Pred	Signif of difference
	x	$s_{\bar{x}}$	$\bar{x}$	$p<$	x	$s_{\bar{x}}$	$\bar{x}$	$p<$
Age	43 0	1 8			42 6	2 6		
Height	162 1	1 2			166 8	2 [illegible]		
Weight	61 6	2 3			61 0	1 3		
TLC	4 36	0 15	5 06	0 001	4 46	0 39	[illegible] 26	—
RI	1 52	0 06	1 [illegible]4	—	1 [illegible]8	0 08	1 75	—
RV	1 [illegible]2	0 06	1 33	0 05	1 58	0 08	1 29	0 01
FRC	2 34	0 09	2 56	—	2 45	0 18	2 83	—
VC	2 84	0 15	3 39	[illegible] 001	2 [illegible]	0 32	3 72	0 0[illegible]
FEV$_1$	2 19	0 12	2 66	0 005	2 12	0 30	2 94	0 02
FEV$_1$%	76 6	1 3	78 2	—	72 3	3 3	75 8	—
*IEF**	4 51	0 27	5 29	0 0[illegible]	4 [illegible]8	0 84	5 75	—
PEF	4 [illegible]1	0 27	4 52	—	4 [illegible]6	0 84	4 57	—
MEF 50%*	2 [illegible]1	0 24	3 6[illegible]	0 005	2 32	0 67	3 93	0 03
MEF 50%	2 71	0 24	3 11	—	2 32	0 67	3 15	—
MEF 25%	1 00	0 11	1 53	0 001	0 79	0 19	1 6	0 001
*Cdyn(l)**	0 115	0 007	0 154	0 001	0 133	0 019	0 158	—
Cdyn(l)	0 11[illegible]	0 007	0 139	0 01	0 133	0 019	0 136	—
f	13 3	1 0			14 4	2 8		
*Gi(l)**	0 43	0 04	0 77	0 001	0 34	0 10	0 [illegible]7	0 001
Gi(l)	0 43	0 04	0 59	0 00[illegible]	0 34	0 10	[illegible] 60	0 0[illegible]

* Predicted value based on predicted *TLC* or *VC*

Table 1[illegible] Men with rheumat c valvular disease Functional class related to values for lung mechan cs

	Funct onal class I (n=6)				Funct onal class II (n=)				Funct onal class III+IV (n=4)			
	Observed		Pred	S gn f of diff	Observed		Pred	S gnif of diff	Observed		Pred	S gnif of diff
	x	s_x	x	$p<$	x	s_x	x	$p<$	x	s_x	x	$p<$
Age	36 3	4 3			48 9	[illegible]			51 3	[illegible] 5		
He ght	1 6 1	[illegible] 9			1	1 6			1 [illegible]	0 6		
Weight	0 3	5 8			3 4	3 3			0 3	5 0		
TLC	6 30	0 [illegible]	[illegible] 04	0 05	5 48	0 [illegible]	6 6	0 001	4 9	0 66	[illegible]	0 01
RV	1 3	0 0	1 69	—	1 4	0 10	1 5[illegible]	—	1 61	0 [illegible]7	1 90	—
RV	1 3	0 [illegible]0	1 43	—	1 4	0 10	1 39	0 00[illegible]	1 61	0 [illegible]	1 19	—
FRC	3 [illegible]4	0 [illegible]7	3 64	—	[illegible] 84	0 19	3 4[illegible]	0 0	[illegible]	0 30	3 41	0 0[illegible]
VC	4 5	0 [illegible]9	5 3	0 0	3 4	0 [illegible]1	4 88	0 001	3 18	0 40	4 [illegible]	0 001
FEV$_1$	3 9	0 36	4 15	—	[illegible] 66	0 14	3 4	0 001	2 1[illegible]	0 14	3 68	0 001
FEV$_1$%	8 [illegible]	3 0	8 6	—	4 9	[illegible]	[illegible]7 3	—	63 3	4	[illegible] 0	—
PEF	6 60	0 [illegible]	8 0	0 00[illegible]	6 43	0 6[illegible]	8 06	0 0[illegible]	5 3	0 60	[illegible]	0 0[illegible]
MEF[illegible]0%	4 5[illegible]	0 31	5 33	0 0[illegible]	3 05	0 30	4 69	0 001	[illegible]	0 46	4 5	0 00[illegible]
MEF [illegible]%	1 9[illegible]	0 [illegible]6	1 96	—	1 [illegible]	0 3	1 6	—	0 96	0 18	1 55	0 01
Cdyn(l)	0 1 5	0 019	0 [illegible]19	0 0[illegible]	0 16	0 01[illegible]	0 21	0 00[illegible]	0 109	0 03	0 [illegible]	0 00[illegible]
Cdyn(l)	0 1 5	0 019	0 1 6	—	0 167	0 015	0 156	—	0 109	0 03	0 1[illegible]9	—
f	11 5	[illegible]			8	1 6			15 [illegible]	1 8		
Gt(l)	0 6[illegible]	0 09	1 04	0 001	0 5	0 06	0 9[illegible]	0 001	0 4[illegible]	0 06	0 94	0 001
Gt(l)	0 6[illegible]	0 09	0 89	0 05	0 5	0 03	0 3	—	0 45	0 06	0 6[illegible]	0 0[illegible]

* Pred cted value based on predicted *TLC* or *VC*

Table 13 Women with rheumatic valvular disease Functional class related to values for lung mechanics

	Functional class I ($n=3$)				Functional class II ($n=15$)				Functional class III+IV ($n=8$)			
	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$
Age	40.3	3.9			47.[illegible]	2.4			51.6	2.9		
Height	67.3	3.7			161.[illegible]	1.7			160.9	1.5		
Weight	74.0	6.6			63.3	2.4			53.6	4.1		
TLC	5.37	0.51	5.30	—	4.42	0.18	4.87	0.0[illegible]	3.86	0.13	4.81	0.001
*RV**	1.42	0.13	1.52	—	1.47	0.09	1.48	—	1.65	0.09	1.52	—
RV	1.42	0.13	1.54	—	1.47	0.09	1.34	—	1.65	0.09	1.[illegible]2	0.005
FRC	2.[illegible]1	0.61	2.6[illegible]	—	2.32	0.12	2.[illegible]1	—	2.23	0.06	2.62	0.001
VC	3.95	0.38	3.78	—	2.96	0.16	3.38	0.0[illegible]	2.22	0.11	3.27	0.001
FEV_1	3.13	0.2	2.99	—	2.28	0.13	2.66	0.02	1.66	0.12	2.55	0.001
$FEV_1\%$	79.8	4.4	79.1	—	77.1	1.7	78.3	—	74.5	2.3	77.9	—
PEF	6.03	0.74	5.84	—	4.73	0.34	[illegible].2	—	3.54	0.25	5.12	0.001
PEF	6.08	0.74	6.07	—	4.73	0.34	4.69	—	3.54	0.[illegible]	3.65	—
*MEF*50%*	3.97	0.71	3.98	—	2.84	0.31	3.61	0.05	1.99	0.28	3.51	0.001
*MEF*50%	3.97	0.71	4.14	—	2.84	0.31	3.22	—	1.99	0.28	2.[illegible]3	—
*MEF*25%	1.69	0.46	1.73	—	1.08	0.12	1.54	0.00[illegible]	0.58	0.13	1.43	0.001
*Cdyn(l)**	0.143	0.018	0.158	—	0.122	0.010	0.133	0.005	0.092	0.009	0.154	0.001
Cdyn(l)	0.143	0.018	0.162	—	0.122	0.010	0.142	—	0.092	0.009	0.125	0.00[illegible]
f	9.7	1.7			13.0	1.2			15.4	1.9		
*Gi(l)**	0.56	0.17	0.68	—	0.43	0.05	0.64	0.001	0.40	0.07	0.63	0.005
Gi(l)	0.56	0.17	0.68	—	0.43	0.05	0.60	0.005	0.40	0.07	0.55	—

* Predicted value based on predicted *TLC* or *VC*

Table 14 Men with rheumat c val ular disease Pulmonary roentgenograms related to values for lung mechan cs

	Pulmonary f ndings grade 0–1 ($n = 9$)				Pulmonary f ndings grade [illegible] 4 ($n = 6$)			
	Observed		Pred	S gn f of d fference	Observed		Pred	S gn f of difference
	x	s_x	x	$p<$	x	s_x	x	$p<$
Age	45 0	4 4			4[illegible] 5	[illegible] 9		
He ght	1 [illegible]	1 0			1 7 0	[illegible] 7		
We ght	63 9	[illegible]			9 0	4 4		
TLC	5 8[illegible]	0 [illegible]6	6 [illegible]6	0 01	5 63	0 44	[illegible]	0 005
RV	1 [illegible]3	0 1[illegible]	1	—	1 [illegible]1	0 13	1 91	—
RV	1 [illegible]3	0 16	1 4[illegible]	—	1 [illegible]1	0 12	1 3	0 0[illegible]
FRC	[illegible] 0	0 [illegible]3	3 4	—	[illegible] 53	0 [illegible]8	3 09	0 01
VC	4 15	0 [illegible]5	4 94	0 01	3 02	0 25	[illegible]	0 001
FEV$_1$	3 19	0 33	3 63	—	[illegible] 88	0 [illegible]5	[illegible] 96	0 001
FEV$_1$%	8[illegible] 9	[illegible] 6	8[illegible] 0	—	[illegible]5 5	[illegible]6	[illegible]	—
PEF	6 56	0 63	[illegible]91	—	6 30	0 31	8 8	0 001
*MEF*50%	3 [illegible]	0 39	4 69	0 0[illegible]	3 54	0 30	4 86	0 00[illegible]
MEF[illegible]5%	1 [illegible]0	0 30	1 [illegible]	—	1 [illegible]9	0 [illegible]0	1 [illegible]1	—
Cdyn(l)	0 169	0 013	0 [illegible]16	0 00[illegible]	0 14	0 0[illegible]8	0 [illegible]33	0 00[illegible]
Clyn(l)	0 169	0 013	0 [illegible]1	—	0 147	0 0[illegible]8	0 [illegible]9	—
f	9 0	1 4			14 5	[illegible]		
Gt(l)	0 33	[illegible]06	0 96	0 001	0 83	0 10	1 0[illegible]	0 00[illegible]
G (l)	0 53	0 08	0 61	0 0	0 62	0 10	0 [illegible]6	—

* Pred cted value based on pred cted *TLC* or *VC*

Table 1[illegible] Women with rheumatic valvular disease Pulmonary roentgenograms related to values for lung mechanics.

	Pulmonary findings grade 0–1 ($n=10$)				Pulmonary findings grade 2–4 ($n=16$)			
	Observed		Pred.	Signif of difference	Observed		Pred.	Signif of difference
	x	$s_{\bar{x}}$	$\hat{x}$	$p<$	x	$s_{\bar{x}}$	x	$p<$
Age	44.[illegible]	3 5			50 0	1 56		
Height	164 [illegible]	2 0			160 8	1 44		
Weight	62.[illegible]	2 [illegible]			61 0	3 4		
TLC	4 93	0 [illegible]6	5 06	—	4 01	0 12	4 50	0 001
*RV**	1 [illegible]2	0 11	1 [illegible]0	—	1 [illegible]2	0 0[illegible]	1 49	—
RV	1 [illegible]2	0 11	1 46	—	1 [illegible]2	0 07	1 24	0 00[illegible]
FPC	2 [illegible]3	0 18	2 66	—	2 21	0 10	2 [illegible]0	0 0[illegible]
VC	3 41	0 26	3 [illegible]	—	2 49	0 11	3 29	0 001
FEV$_1$	2.67	0 21	2 60	—	1 69	0 10	2 [illegible]8	0 001
FEV$_1$%	[illegible]8 3	2 0	[illegible]8 6	—	75 [illegible]	1 7	78 1	—
*PEF**	[illegible] 48	0 41	5 [illegible]1	—	3 91	0 26	4 08	—
PEF	[illegible] 48	0 41	[illegible] 32	—	3 91	0 26	4 08	—
MEF[illegible]0%*	3 44	0 41	3 [illegible]7	—	2 26	0 23	3 [illegible]3	0 001
MEF[illegible]0%	3 44	0 41	3 64	—	2 26	0 23	2 78	—
*MEF*2[illegible]%	1 2[illegible]	0 18	1 62	—	0 84	0 13	1 4[illegible]	0 001
Cdyn(l)	0 141	0 012	0 1[illegible]	—	0 099	0 006	0 1[illegible]3	0 001
Cdyn(l)	0 141	0 012	0 1[illegible]2	—	0 099	0 006	0 131	0 001
f	12 4	1 4			13 9	1 3		
Gl(l)	0 [illegible]	0 0	0 6[illegible]	—	0 36	0 04	0 63	0 001
Gl(l)	[illegible] [illegible]	0 0[illegible]	0 64	—	0 36	0 04	0 [illegible]6	0 001

Predicted value based on predicted *TLC* or *VC*

Table 16 Twenty six women with rheumatic valvular disease. Multiple regression equations with age (A) h[illegible] in cm, weight (W) in kg lung volumes roentgenographic heart volume (ml) and hemodynamic va[illegible] independent variables Only significant ($p<0.05$) equations given Standard errors of regression coef[illegible] parentheses

Eq.no	Sex	Dependent variable	Independent variables	Constant	PS.
1	F	TLC =	+0.0806 H (±0.01949)	−8.[illegible]8	0.5[illegible]
2	F	TLC =	+0.0[illegible]043 H+0.0211 W (±0.01582) (±0.0090)	−8.351	0.[illegible]
3	F	TLC =	+0.0[illegible]094 H+0.0241 W−0.000930 RHV (±0.0164[illegible]) (±0.0088) (±0.0003[illegible])	−[illegible]6.8	0.4[illegible]
4	F	TLC =	−0.0[illegible]84 H −0.016[illegible] $\bar{P}_{PA}$ (±0.01[illegible]6) (±0.0063)	−7.[illegible]0	0.5[illegible]
5	F	TLC =	+0.08044 H −0.0[illegible]9 PVR (±0.01[illegible]56) (±0.023[illegible])	−8.426	0.[illegible]
6	F	RV =	+0.02[illegible]46 A+0.[illegible] TLC (±0.00481) (±0.0[illegible]0)	−0.801	0.2[illegible]
7	F	FRC =	+0.0[illegible]440 H (±0.01410)	−3.240	0.4[illegible]
8	F	VC =	−0.02[illegible]44 A−0.0181[illegible] H+0.02449 W (±0.01042) (±0.0180[illegible]) (±0.00[illegible]8)	−4.[illegible]0	0.4[illegible]
9	F	VC =	−0.02[illegible]39 A+0.0343 H+0.0[illegible]16 W−0.000[illegible]3 RHV (±0.00984) (±0.01413) (±0.00680) (±0.0002[illegible]0)	−5.[illegible]44	0.[illegible]
10	F	VC =	−0.02[illegible]4 A+0.049[illegible] H+0.0[illegible]0 W−0.01[illegible]8 $\bar{P}_{PA}$ (±0.00941) (±0.014[illegible]) (±0.00[illegible]) (±0.00[illegible]1)	−4.593	0.3[illegible]
11	F	VC =	−0.0[illegible]94 A+0.05320 H+0.0[illegible] W−0.06[illegible] PVR (±0.0098) (±0.014[illegible]1) (±0.00[illegible]3) (±0.0[illegible]60)	−5.[illegible]1	0.3[illegible]
12	F	VC =	−0.02[illegible] A−0.[illegible]942 TLC (±0.0016) (±0.0550)	−0.801	0.20
13	F	VC =	−0.0159 $\bar{P}_{PA}$ (±0.00[illegible])	−3.382	0.69
14	F	VC =	−0.0843 PVR (±0.0431)	−3.[illegible]1	0.67
15	F	FEV_1 =	−0.0[illegible]81 A+0.049[illegible] H (±0.0106) (±0.01[illegible]9)	−4.4[illegible]	0.44
16	F	FEV_1 =	−0.02[illegible] A+0.02[illegible]3[illegible] H+0.0[illegible]32 W (±0.008[illegible]) (±0.01343) (±0.0065)	−3.40[illegible]	0.36
17	F	FEV_1	−0.02[illegible]8 A+0.03981 H+0.0238 W−0.0003[illegible] RHV (±0.0084) (±0.01[illegible]) (±0.0060) (±0.000[illegible])	−3.963	0.3[illegible]
18	F	FEV_1	−0.026[illegible] A+0.04880 H −0.0116 $\bar{P}_{PA}$ (±0.0096) (±0.0146[illegible]) (±0.0053)	−4.136	0.41
19	F	FEV_1 =	−0.0[illegible]91 A+0.04616 H −0.02[illegible]3 $\bar{P}_{LA}$ (±0.0096) (±0.0142[illegible]) (±0.0104)	−3.[illegible]4	0.40
20	F	PEF =	−0.0[illegible]488 A (±0.0261[illegible])	+8.20[illegible]	1.1[illegible]
21	F	PEF =	+0.09901 H (±0.04043)	−11.[illegible]34	1.23[illegible]

Eq no	Sex	Dependent variable	Independent variables	Constant	PSD	R^2
22	F	$PEF =$	$+1.3610\ TC$ (± 0.2495)	$+0.644$	0.926	0.55
23	F	$PEF =$	$+1.0728\ TLC$ (± 0.2876)	-0.165	1.103	0.37
24	F	$\dot{V}EF50\% =$	$+0.6530\ A$ (± 0.0223)	$+5.844$	1.069	0.25
25	F	$\dot{V}EF50\% =$	$+0.7445\ TLC$ (± 0.2831)	-0.584	1.085	0.22
26	F	$\dot{V}EF50\% =$	$+1.1259\ TC$ (± 0.2304)	-0.489	0.889	0.35
27	F	$\dot{V}EF25\% =$	$-0.02338\ A - 0.02[illegible]1\ \bar{P}_{LA}$ $(\pm 0.01105)\ (\pm 0.0128)$	$+2.591$	0.505	0.28
28	F	$\dot{V}EF25\% =$	$+0.04692\ H$ (± 0.01640)	-0.610	0.502	0.25
29	F	$\dot{V}EF25\% =$	$+0.04380\ H - 0.0090\ \bar{P}_{PA}$ $(\pm 0.01410)\ (\pm 0.0001)$	-0.135	0.492	0.32
30	F	$\dot{V}EF25\% =$	$+0.04440\ H - 0.0241\ \bar{P}_{LA}$ $(\pm 0.01410)\ (\pm 0.0100)$	$-5.[illegible]5$	0.474	0.36
31	F	$\dot{V}EF25\% =$	$+0.3992\ TLC$ (± 0.1282)	-0.744	0.491	0.29
32	F	$\dot{V}EF25\% =$	$+0.5149\ TC$ (± 0.1164)	-0.468	0.432	0.45
[illegible]	F	$Cdyn(l) =$	$+0.00216\ H - 0.00[illegible]64\ PVR$ $(\pm 0.001028)\ (\pm 0.00198)$	-0.2198	0.0218	0.35
34	F	$Cdyn(l) =$	$+0.02884\ TLC$ $(\pm 0.008[illegible]1)$	-0.0104	0.0315	0.34
35	F	$Cdyn(l) =$	$+0.28446\ TC$ (± 0.00867)	$+0.0345$	0.0322	0.31
36	F	$Cdyn(l) =$	$-0.00108\ \bar{P}_{PA}$ (± 0.00044)	$+0.1462$	0.0346	0.21
37	F	$Cdyn(l) =$	$-0.00567\ PVR$ (± 0.00212)	$+0.1819$	0.0340	0.23
38	F	$Gt(l) =$	$+0.01819\ H$ (± 0.00550)	-2.514	0.108	0.31
39	F	$Gt(l) =$	$+0.1540\ TLC$ $(\pm 0.042[illegible])$	-0.238	0.164	0.35
40	F	$Gt(l) =$	$+0.1883\ TLC + 0.0044\ \bar{P}_{PA}$ $(\pm 0.0433)\ (\pm 0.0021)$	-0.312	0.133	0.45
41	F	$Gt(l) =$	$-0.1869\ TLC + 0.0[illegible]15\ PVR$ $(\pm 0.0429)\ (\pm 0.010[illegible])$	-0.444	0.153	0.46
42	F	$Gt(l) =$	$+0.1485\ TC$ (± 0.0456)	$+0.011$	0.169	0.31
43	F	$Gt(l) =$	$+0.1893\ TC + 0.0231\ PVR$ $(\pm 0.0466)\ (\pm 0.0108)$	-0.172	0.156	0.41

Table 17 Seventeen men with rheumatic valvular disease. Multiple regression equations with age (A), height ([illegible] cm, weight (W) in kg, lung volumes, roentgenographic heart volume (ml) and hemodynamic variables [illegible] dependent variables. Except in eq.s nos 8–10 only significant ($p < 0.05$) equations given. Standard err[illegible] regression coefficients in parentheses

Eq.no	Sex	Dependent variable	Independent variables	Constant	RSD
1	M	TLC =	−0 0[illegible]6 H −0 2[illegible] PVR (±0 03[illegible]6) (±0 09668)	−7 [illegible]	0 807
2	M	TLC =	−0 24497 PVR (±0 106[illegible]6)	+6 144	0 891
3	M	RV =	−0 0[illegible]358 A +0 29[illegible] TLC (±0 00 3[illegible]) (±0 0 23)	−1 011	0 [illegible] 0
4	M	VC =	−0 04122 A +0 06669 H −0 02 1[illegible] $\bar{P}_{PA}$ (±0 01[illegible]) (±0 029[illegible]) (±0 003[illegible]9)	−5 29[illegible]	0 [illegible]90
5	M	VC =	−0 04009 A −0 064 8 H −0 19[illegible] PVR (±0 0144) (±0 02[illegible]) (±0 068[illegible])	−5 [illegible]	0 [illegible]
6	M	VC =	−0 0[illegible]358 A −0 012 TLC (±0 00 34) (±0 0 [illegible]3)	−1 011	0 2 0
[illegible]	M	VC =	−0 [illegible]104 PVR (±0 088[illegible])	−4 361	0 [illegible]40
8	M	FEV$_1$ =	−0 0[illegible] A −(0 04 38 H) −0 0[illegible]146 $\bar{P}_{PA}$ (±0 01666) (±0 03160) (±0 00960)	−2 48[illegible]	0 638
9	M	FEV$_1$ =	−0 05284 A ±(0 04982 H) −0 048[illegible]4 $\bar{P}_{PA}$ (±0 01685) (±0 0321) (±0 0[illegible]19)	−2 [illegible] 2	0 646
10	M	FEV$_1$ =	−0 05116 A +(0 04462 H) −0 1[illegible] PVP (±0 01694) (±0 03198) (±0 0 8)	−2 24[illegible]	0 64
11	M	FEV$_1$ =	−0 0401[illegible] A +0 4946 TLC (±0 01542) (±0 1[illegible]16)	−1 925	0 56[illegible]
12	M	FEV$_1$% =	−0 39490 $\bar{P}_{LA}$ (±0 [illegible]825)	−83 [illegible]	8 44
13	M	Cdyn(l) =	−0 04316 VC (±0 0128[illegible])	−0 0144	0 04[illegible]
14	M	Cdyn(l) =	−0 039 3 TLC (±0 00939)	−0 06[illegible]8	0 038[illegible]
15	M	Gi(l) =	−0 00033[illegible] RHV (±0 0001[illegible]0)	−0 [illegible]23	0 1[illegible]4
16	M	R C =	−0 000[illegible] RHV (±0 000010)	−0 5684	0 1164

Table 18 Men with rheumatic valvular disease Mean pressure in pulmonary artery ($\overline{P}_{PA}$) related to values fo lung mechanics

	$\overline{P}_{PA} \leq 19$ mm Hg (n=6)				$\overline{P}_{PA}$=20–29 mm Hg (n=9)				$\overline{P}_{PA} \geq 30$ mm Hg (n=2)			
	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	p<	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	p<	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	p<
Age	39.2	4.9			49.0	2.6			44.?	0.?		
Height	172.0	1.3			175.0	2.1			174.?	1.?		
Weight	71.0	2.6			72.0	3.9			71.?	15.5		
TLC	5.6	0.41	6.?1	0.0?	5.66	0.31	6.9?	0.001	5.18	1.40	6.91	—
RV*	1.?2	0.09	1.63	—	1.82	0.1?	1.9?	—	1.74	0.42	1.81	—
RV	1.?2	0.09	1.16	0.02	1.82	0.1?	1.45	0.05	1.74	0.42	1.19	—
FRC	2.?8	0.13	3.35	0.01	2.94	0.26	3.??	0.0?	3.14	0.48	3.?4	—
VC	4.15	0.43	5.00	—	3.63	0.21	5.03	0.001	3.44	0.9?	5.0?	—
FEV_1	3.2?	0.?2	3.93	—	2.85	0.1?	3.63	0.001	2.24	0.50	3.91	0.00?
$FEV_1\%$	60.9	3.6	79.3	—	74.8	2.?	76.6	—	66.3	4.4	77.6	0.??
PEF	6.4?	0.?1	8.56	0.02	6.19	0.56	7.91	0.01	5.?0	0.68	8.21	0.(
$MEF50\%$	3.9?	0.56	5.18	0.0?	3.49	0.??	4.69	0.00?	2.30	0.2?	4.92	0.0
$MEF25\%$	1.98	0.40	1.68	—	1.24	0.16	1.61	—	0.89	0.21	1.?4	0.0(
$Cdyn(l)$	0.16?	0.02?	0.20?	—	0.149	0.013	0.229	0.001	0.1??	0.084	0.2?2	—
$Cdyn(l)$	0.165	0.025	0.158	—	0.149	0.013	0.161	—	0.15?	0.084	0.129	—
f	9.?	2.2			11.1	1.?			17.5	0.5		
$Gt(l)$*	0.5?	0.09	0.98	0.001	0.61	0.0	0.98	0.001	0.39	0.00	0.99	0.001
$Gt(l)$	0.5?	0.09	0.81	0.0?	0.61	0.0?	0.?4	—	0.39	0.00	0.6?	0.001

* Predicted value based on predicted TLC or VC

Table 19 Women with rheumatic valvular disease. Mean pressure in pulmonary artery ($\bar{P}_{PA}$) related to values for lung mechanics.

	$P_{PA} \leq 19$ mm Hg (n = 10)				P_{PA} = [illegible]–[illegible]9 mm Hg (n = [illegible])				$P_{PA} \geq 30$ mm Hg (n = [illegible])			
	Observed		Pred	Sign f of diff	Observed		Pred	Sign f of diff	Observed		Pred	Sign f of diff
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$
Age	4[illegible] [illegible]	3 1			[illegible]0 8	3 4			4[illegible] 9	[illegible] 9		
Height	163 [illegible]	[illegible] 1			16[illegible] 1	[illegible]			160 3	1		
Weight	[illegible]4 1	4 0			63 8	[illegible] 3			[illegible]6 3	4 9		
TLC	4 6	0 [illegible]5	5 01	—	4 46	0 [illegible]9	4 90	—	3 [illegible]	0 17	4 [illegible]6	0 001
RV	1 49	0 10	1 5[illegible]	—	1 6[illegible]	0 1[illegible]	1 [illegible]3	—	1 41	0 08	1 4[illegible]	—
PV	1 49	0 10	1 4[illegible]	—	1 8[illegible]	0 1[illegible]	1 59	—	1 41	0 08	1 15	0 01
FRC	2 38	0 1[illegible]	[illegible] 80	—	[illegible] 48	0 [illegible]	[illegible]	—	2 14	0 08	3 54	0 001
VC	3 18	0 [illegible]4	3 47	—	2 81	[illegible]	3 34	—	[illegible] 46	0 [illegible]0	3 34	0 001
FEV,	[illegible] 30	0 [illegible]	2 [illegible]3	—	[illegible] 11	0 19	[illegible]	0 0[illegible]	1 89	0 [illegible]0	[illegible] 83	0 00[illegible]
FEV$_1$%	78 0	1	79 3	—	[illegible] 4	[illegible] 8	8 0	—	6 0	[illegible] 4	[illegible]	—
*PEF**	4 8	0 4	[illegible] 30	—	4 58	0 49	5 [illegible]	—	4 00	0 40	[illegible]	0 0[illegible]
PEF	4 8	0 4	4 09	—	4 58	0 49	4 48		4 00	0 40	3 98	—
MEF[illegible]0%	3 [illegible]9	0 44	3 89	—	[illegible]	0 [illegible]3	3 5	0 001	[illegible] 41	0 43	3 [illegible]	0 0[illegible]
MEF[illegible]0%	3 [illegible]9	0 44	3 4[illegible]	—	[illegible]	0 [illegible]2	3 08	0 01	[illegible] 41	0 43	[illegible] 6	—
MEF[illegible]0%	1 [illegible]8	0 [illegible]1	1 54	—	0 83	0 09	1 45	0 001	0 81	0 [illegible]1	1 38	0 00[illegible]
Cdyn(l)	0 13[illegible]	0 013	0 1[illegible]3	0 0[illegible]	0 1[illegible]	0 006	0 15[illegible]	0 001	0 0[illegible]8	0 013	0 131	0 001
Cdyn(l)	0 13[illegible]	0 013	0 148	—	0 1[illegible]	0 006	0 140	0 0[illegible]	0 088	0 013	0 1	0 01
f	11 [illegible]	0 8			11 8	1			1[illegible] 6	[illegible]		
Gi(l)	0 46	0 0[illegible]	0 6[illegible]	0 01	0 41	0 09	0 64	0 0[illegible]	0 43	0 0	0 63	0 0
Gi(l)	0 46	0 06	0 6[illegible]	0 0	0 41	0 09	0 60	0 0[illegible]	0 43	0 0	0 5[illegible]	—

* Predicted value based on predicted *TLC* or *VC*

Table 20 Men with rheumatic valvular disease Mean pressure in left atrium ($\bar{P}_{LA}$) related to values for lung mechanics

	$\bar{P}_{LA} \leq 9$ mm Hg ($n=6$)				$\bar{P}_{LA}=10$–19 mm Hg ($n=8$)				$\bar{P}_{LA} \geq 20$ mm Hg ($n=3$)			
	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$
Age	33.2	5.0			50.8	2.4			41.0	3.3		
Height	173.3	1.0			173.5	2.5			176.0	1.7		
Weight	72.3	2.7			71.9	4.4			69.3	9.2		
TLC	5.85	0.36	6.81	0.05	5.38	0.33	6.63	0.001	5.69	0.98	7.04	—
*RV**	1.52	0.09	1.67	—	1.82	0.17	1.92	—	1.[illegible]5	0.24	1.79	—
RV	1.52	0.09	1.32	—	1.82	0.14	1.39	0.05	1.75	0.24	1.30	—
FRC	2.86	0.13	3.46		2.85	0.30	3.47	—	3.10	0.27	3.63	—
VC	4.33	0.36	5.08	—	3.56	0.19	4.90	0.001	3.94	0.[illegible]4	5.23	—
FEV$_1$	3.45	0.47	3.98	—	2.62	0.17	3.73	0.001	2.70	0.54	4.04	0.02
FEV$_1$%	82.3	3.0	78.9	—	74.0	3.1	76.8	—	68.5	3.4	77.8	0.02
PEF	6.97	0.49	8.68	0.01	5.77	0.68	7.77	0.0[illegible]	5.98	0.48	8.25	0.001
*MEF*50%	4.20	0.43	5.18	—	3.16	0.32	4.60	0.001	3.08	0.79	5.09	0.02
*MEF*25%	2.10	0.33	1.88	—	1.09	0.18	1.36	0.05	1.14	0.28	1.82	0.05
Cdyn(l)	0.166	0.025	0.212	—	0.154	0.014	0.226	0.001	0.140	0.051	0.324	—
Cdyn(l)	0.166	0.025	0.169	—	0.154	0.014	0.149	—	0.140	0.051	0.150	—
f	9.8	2.2			11.3	1.9			14.3	3.2		
*Gt(l)**	0.55	0.08	0.99	0.001	0.58	0.08	0.96	0.001	0.56	0.17	1.02	0.02
Gt(l)	0.55	0.08	0.84	0.01	0.56	0.06	0.69	—	0.56	0.17	0.[illegible]	—

* Predicted value based on predicted *TLC* or *VC*

Table 21 Women with rheumatic valvular disease Mean pressure in left atrium ($\bar{P}_{LA}$) related to values for lung mechanics

	$\bar{P}_{LA} \leq$ [illegible] mm Hg (n [illegible] 4)				$\bar{P}_{LA}$ 10–19 mm Hg (n=11)				$P_{LA} \geq$ [illegible]0 mm Hg (n=11)			
	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff	Observed		Pred	Signif of diff
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$p<$
Age	43 8	4 5			51 0	3 0			46 5	2 5		
Height	164 8	2 8			16[illegible]	2 1			161 0	1 7		
Weight	61 8	7 1			65 9	3 0			57 [illegible]	3 6		
TLC	4 57	0 44	5 11	—	4 [illegible]	0 [illegible]5	4 91	—	3 93	0 13	4 81	0 001
RV	1 35	0 19	1 51	—	1 65	0 08	1 [illegible]4	—	1 44	0 09	1 45	—
RV	1 35	0 19	1 34	—	1 65	0 08	1 48	—	1 44	0 09	1 17	0 0[illegible]
FRC	40	0 [illegible]6	[illegible] 70	—	2 49	0 19	[illegible] 49	—	[illegible] 16	0 06	2 57	0 001
VC	3 [illegible]1	0 37	3 60	—	3 07	0 [illegible]5	3 35	—	2 46	0 16	3 36	0 001
FEV_1	[illegible] 53	0 35	2 64	—	2 33	0 [illegible]1	2 62	—	1 93	0 15	2 65	0 001
FEV_1%	8 0	4 3	78 7	—	75 5	2 0	78 0	—	77 [illegible]	1 9	78 4	—
PEF	4 90	1 01	5 [illegible]8	—	4 65	0 36	5 23	—	4 03	0 37	[illegible]	0 01
PEF	4 90	1 01	5 04	—	4 85	0 36	4 84	—	4 03	0 37	4 01	—
*MEF*50%	3 44	0 90	3 8[illegible]		[illegible] 69	0 34	3 58	0 05	[illegible] 47	0 3[illegible]	3 59	0 005
*MEF*50%	3 44	0 90	3 45		[illegible] 69	0 34	3 3[illegible]	—	[illegible] 47	0 3[illegible]	2 77	—
MEF 5%	1 3[illegible]	0 33	1 64		1 04	0 18	1 46	0 05	0 84	0 15	1 [illegible]	0 001
Cdyn(l)	0 104	0 00	0 156	0 001	0 135	0 011	0 155	—	0 100	0 012	0 15[illegible]	0 001
Cdyn(l)	0 104	0 007	0 145	0 001	0 135	0 011	0 148	—	0 100	0 012	0 1[illegible]8	0 05
f	11 0	1 5			11 6	0 9			15 9	1 9		
G[illegible]*(l)*	0 30	0 03	0 66	0 001	0 49	0 07	0 64	0 05	0 40	0 05	0 63	0 001
G (l)	0 39	0 03	0 61	0 001	0 49	0 07	0 6[illegible]		0 40	0 05	0 55	0 02

Predicted value based on predicted *TLC* or *VC*

Table 25 Ten women with rheumatic valvular disease The effect of correcting flow volume curves for alveolar gas compression on the maximum expiratory flow values ($\dot{V}$ *max, LPS*)

$\dot{V}$*max* *LPS*	Per cent of vital capacity 10	20	30	40	50	60	70
Not corrected							
$\bar{x}$	0.15	0.39	0.77	1.45	2.22	2.93	3.26
$s_{\bar{x}}$	0.03	0.08	0.21	0.34	0.45	0.51	0.[illegible]1
Range	0.09–0.40	0.10–0.8[illegible]	0.1[illegible]–1.[illegible]	0.24–3.99	0.42–5.10	0.59–5.[illegible]4	0.88–6.[illegible]2
Corrected							
$\bar{x}$	0.21	0.4[illegible]	0.99	1.76	2.60	3.41	3.80
$s_{\bar{x}}$	0.04	0.10	0.1[illegible]	0.37	0.48	0.[illegible]6	0.5[illegible]
Range	0.04–0.32	[illegible].15–1.00	0.23–2.3[illegible]	[illegible].42–4.35	[illegible].65–5.90	[illegible].97–6.[illegible]	1.45–6.[illegible]5
$\bar{d}$	0.06	0.09	0.22	0.31	0.38	0.48	0.54
Signif of diff $p<$	0.005	0.00[illegible]	0.01	0.01	0.02	0.0[illegible]	0.05

Table 26 Ten women with rheumatic valvular disease Peripheral airway conductance (*Gus LPS*/cm H_2O) in relation to predicted normal values

Gus *LPS*/cm H_2O	Per cent of vital capacity 10	20	30	40	50	60	70
Ten patients with rheumatic valvular disease							
$\bar{x}$	0.12	0.22	0.[illegible]4	0.28	0.32	0.34	0.29
$s_{\bar{x}}$	0.03	0.0[illegible]	0.05	0.07	0.06	0.08	0.06
Pred normal	0.26	0.34	0.46	0.45	0.51*	0.49*	0.4[illegible]*
Signif of diff $p<$	0.05	—	0.01	—	0.02	0.0[illegible]	0.02
Seven patients without cough and sputum 3 months a year							
$\bar{x}$	0.18	0.26	0.28	0.32	0.36	0.39	0.34
$s_{\bar{x}}$	0.0[illegible]	0.09	0.0[illegible]	0.09	0.08	0.08	0.09
Pred normal	0.21	0.28	0.46	0.39	0.50*	0.49	[illegible].46*
Signif of diff $p<$	—	—	0.05	—	—	—	—

* Predicted value based on predicted *VC* When observed values for *VC* were used no significant differences from predicted normal values were found.

GÖTEBORG 1968
ELANDERS BOKTRYCKERI AKTIEBOLAG

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 490

EARLY AND LATE RESULTS OF CONVERSION OF ATRIAL FIBRILLATION WITH QUINIDINE

A clinical and hemodynamic study

BY

GUN CRAMÉR

GÖTEBORG 1968

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 490

From the Department of Medicine I (Professor Lars Werko) Sahlgrenska Hospital University of Goteborg Goteborg Sweden

EARLY AND LATE RESULTS OF CONVERSION OF ATRIAL FIBRILLATION WITH QUINIDINE

A clinical and hemodynamic study

BY

GUN CRAMÉR

GÖTEBORG 1968

From the Swedish by
Stanley H Pretorius
and David H Lewis

ORSTADIUS BOKTRYCKERI AKTIEBOLAG
GÖTEBORG 1968
1 644

To

my parents
and
Kim, Per and Bo

Contents

List of abbreviations

AF = atrial fibrillation
SR = sinus rhythm
RHD = rheumatic heart disease
MS = mitral stenosis
MS op = operated mitral stenosis
MI = mitral regurgitation
AS = aortic stenosis
AI = aortic regurgitation
CHD = coronary heart disease
HHD = hypertensive heart disease
RBBB = right bundle branch block
LBBB = left bundle branch block
ES = extra systoles
A = atrial
V = ventricular
CO = cardiac output
HR = heart rate
SV = stroke volume
O_2 cons = O_2 consumption
BA_S BA_D BA_M = systolic, diastolic and mean pressure in the brachial artery
A-V diff = arterio-venous O_2 difference
DC = direct current
BSA = body surface area
l = liter
mEq = milli equivalents
kpm/min = kilopondmeter per minute

SE = standard error

Introduction

A method for converting atrial fibrillation to normal sinus rhythm by means of direct current (DC) countershock was introduced by Lown *et al* 1962[a,b] Since it thus became possible to convert atrial fibrillation to sinus rhythm quickly simply, safely and with a high conversion frequency, Lown *et al*, 1963, the advantages and disadvantages of conversion had to be re-evaluated

Prior to this many authors had expressed doubt as to the value of the conversion of atrial fibrillation with quinidine, primarily because of the fear of complications in the form of life threatening arrhythmias caused by quinidine or arterial emboli at the time of conversion to sinus rhythm. Often this led to the careful use of small doses of quinidine Furthermore, the advantage of conversion to sinus rhythm over simply digitalization of the patient with atrial fibrillation so as to control the ventricular rate was questioned No large, Swedish series including follow-up in which the patients had been converted with quinidine alone has ever been published

The present investigation was carried out since Sokolow and Ball, 1956 had shown that there was a small risk of emboli in connection with quinidine conversion and that there was a relationship between high quinidine concentrations in plasma and severe toxic side effects

A quinidine determination method of the same type as the one used by Sokolow made it possible to use high quinidine doses with a greater degree of safety than was previously possible Brodie *et al*, 1947[a,b] Edgar and Sokolow, 1950 Sokolow and Edgar, 1950, Cramer and Isaksson, 1963

The present paper comprises 237 patients in whom conversion attempts were made with quinidine prior to the introduction of DC countershock Of these 237 cases, 234 have been followed for at least 28 months up to 8 years or until they died These data permit a comparison with other similar quinidine- or DC countershock-converted patient series with regard to conversion frequency and side effects It also illustrates the value of a long observation period after conversion attempts This is of great importance, regardless of which method is used for conversion to sinus rhythm

Some questions of practical significance were

- Is the patient subjectively or hemodynamically improved after conversion to normal sinus rhythm?
- What criteria should be used in the selection of cases for conversion?
- Is there any patient group which is less likely to maintain sinus rhythm?
- Has quinidine any importance for the maintenance of sinus rhythm or not?
- Is there a reduced risk of emboli with sinus rhythm?
- What are the risks of using quinidine as a conversion agent and as a prophylactic against fibrillation?

Survey of literature

A. Quinidine conversion

Since Wenckebach, 1914, showed that it was possible to convert atrial fibrillation to sinus rhythm in man with quinine, and Frey, 1918[a], found that its isomer quinidine was the most effective antiarrhythmic of the cinchona alkaloids many authors have expressed their views on the conversion to atrial fibrillation with quinidine as follows

Conversion frequency

The conversion frequency from atrial fibrillation to sinus rhythm with quinidine has varied considerably between 30 and 88%, in different patient series See Table 1 a.

As for the sex of the patient women seem to be more easily converted than men but do not maintain their sinus rhythm as well Viko *et al*, 1923 Friedberg and Sjoestroem 1956, however reported a conversion frequency which was twice as high for men than for women

The age of the patient has no influence on the conversion results according to Frey 1921 Hewlett and Sweeney 1921 Viko *et al* 1923 Wolff and White 1929 Laake 1945 Mosbech and Thomsen 1959 and Rokseth 1963

Between different diagnostic groups there have been definite variations in the conversion frequency McMillan and Welfare 1947 were of the opinion that the best results were obtained when the patient with atrial fibrillation had heart disease that was rheumatic in origin Goldman, 1959, on the other hand, reported a conversion frequency of 80—87% for atherosclerotic heart disease and 50—55% for mitral stenosis Sandoe *et al* 1965 reported that atrial fibrillation on a nonrheumatic basis was more easily converted than when there was a rheumatic origin. When the χ^2 test is applied to the figures of Sandoe *et al* the difference is significant $p < 0.001$

A long duration of atrial fibrillation, i e more than one year has an unfavourable effect on the conversion frequency according to Frey 1921 This was confirmed by Hewlett and Sweeney in 1921 Viko *et al*, 1923 considered atrial fibrillation for more than 2 years the major factor in preventing a good result with quinidine therapy both with respect to the conversion frequency and the maintenance of sinus rhythm Maurice *et al* 1956, and Rokseth, 1963 also showed that a long history of fibrillation had a negative influence on the results of the conversion

Gerstenblith *et al*, 1966 showed 85 per cent conversion frequency in elderly

Table 1 a Series with quinidine treatment

Authors		No of patients treated	No of patients in sinus rhythm	Sinus rhythm % of patients treated	Follow up period months	Quinidine maintenance therapy	No of restored patients followed	No of patients in sinus rhythm at end of follow up period
Frey	(1921)	50	21	42	—	—	—	—
Viko *et al*	(1923)	75*	51	68	< 1—10	+	51	26
Hay	(1924)	265	156	59	—	—	—	—
Maynard	(1928)	53	38	72	6	+	38	15
Parkinson and Campbell	(1929)	44	30	68	6	+	30	23
Wolff and White	(1929)	62	42	68	< 4—48	—	38	27
Hall	(1941)	300	?	47†	—	—	—	—
Berman and Blumenthal	(1942)	48	15	31	2—24	?	15	8
Laake	(1945)	34	23	68	3	s w	23	16
					6—120	—	16	11
McMillan and Welfare	(1947)	50	44	88	1—24	+	?	25
Fisherman and Schlesner	(1950)	31	12	39	—	—	—	—
Goldman	(1951)	80	67	84	—	+	—	—
Holzman and Brown	(1951)	57**	30	53	2—16	—	28	14
Yount *et al*	(1952)	155	119	76	1—36	+	41	35
Bedard	(1954)	67	60	69	—	+	—	—
Friedberg and Sjoestroem	(1956)	133	63	47	—	—	—	—
Sokolow and Ball	(1956)	177***	153	86	< 1—49	+	99	35
Maurice *et al*	(1956)	313	257	66	12	+	[illegible]	28
Beckwith *et al*	(1956)	30	21	70	24	+	21	2
Mosbech and Thomsen	(1959)	87	39	45	—	—	—	—
Blondeau *et al*	(1960)	575	?	62†	—	—	—	—
Freeman and Wexler	(1960)	100	57	57	—	—	—	—
Kassane *et al*	(1961)	368	298	81	—	—	—	—
Rokseth and Storstein	(1963)	274	129	47	—	—	—	—
Rokseth	(1963)	200	107	54	6	+	94	65
Sandoe *et al*	(1965)	100	58	58	3—8	—	34	15
Own series	(1968)	237	148	62	48	+	144	25

s w = some weeks

* = including 4 patients with flutter
** = " 2 "
*** = " 8 "

† = calculated on the number of conversion attempts

patients with atrial fibrillation for 6 months or less

Halmos 1966, showed that 6 out of 6 patients with atrial fibrillation of unspecified duration prior to valvulotomy for mitral stenosis reverted to atrial fibrillation within one year in spite of postoperative conversion Patients with postoperative fibrillation of brief duration, however, were easily converted and

maintained the sinus rhythm for a considerably longer period of time Similar results were reported by Sandoe *et al*, 1965

As for the **roentgenological cardiac size**, Goldman, 1951 found no relationship to the conversion frequency Freeman and Wexler 1960, reported that 3 of their patients with largest cardiac size could be converted Sandoe *et al* 1965, maintained that hearts which are less than 500 ml/m² are easier to convert than hearts larger than 700 ml/m² body surface area (BSA) When χ^2 test is applied to the figures of Sandoe *et al* however there is no statistically significant difference $\chi^2 = 1.558$

Viko *et al* 1923 considered cardiac **decompensation** a limiting factor in quinidine therapy In patients treated for heart failure, the conversion frequency was good but the patients did not maintain the sinus rhythm as well as patients not treated for decompensation According to Maynard 1928 there was a lower conversion frequency in patients with cardiac decompensation

As for cases of atrial fibrillation with **bundle branch block**, Goldman 1951 was of the opinion that quinidine was not contraindicated He had 9 cases in which the QRS interval did not increase after quinidine therapy He gave however no information on the frequency of conversion to sinus rhythm. Freeman and Wexler 1960 succeeded in converting 3 of 5 patients with atrial fibrillation and bundle branch block According to Di Palma 1950 however bundle branch block was an important contraindication for conversion attempts with quinidine

Conversion from atrial fibrillation via flutter to sinus rhythm was found in 65% of the converted cases, Goldman 1951, and in 5% in a series described by Yount *et al*, 1952

Complications and side effects

The risk of **arterial emboli** during conversion with quinidine seemed to be small even before the introduction of anticoagulant therapy Viko *et al*, 1923 reported 3.1% emboli in 71 quinidine-treated patients Hay, 1924 described a series of 286 patients with atrial arrhythmias of whom 265 had atrial fibrillation In these, there were 7 cases of emboli in connection with the quinidine therapy, i e in 2% Hall 1941 reported 3% emboli in a series of 337 cases of quinidine treatment Bedard 1954, found 3 cases of emboli in conversion attempts without previous anticoagulant treatment in 67 patients, i e in 4%

According to Maurice *et al*, 1956, however, the risk of emboli is 10 times greater without than with preceding anticoagulant treatment in quinidine conversion The risk of emboli in conversion attempts with quinidine proved to be very small about 1%, in a series of 177 patients although only selected cases with a recent embolus had been treated with anticoagulants, Sokolow and Ball, 1956 Rokseth 1963 had no embolic episodes in 200 patients treated with anticoagulants

The incidence of **syncope** was 12 out of 274 patients 4.4%, in a series reported by Rokseth and Storstein 1963 and 5 of 80 in the series described by Hamfelt

and Malers 1963 The quinidine syncope, according to these authors seems to be independent of the size of the quinidine dose The underlying mechanism was assumed to be either cardiac with asystole or ventricular fibrillation, Selzer and Wray, 1964 or central with respiratory paralysis Lyon and De Graff 1965, Weisman, 1966

The mortality rate during quinidine treatment was reported to be 3 3%, Thomson, 1956, and between 1 and 4% of the cases, Davies *et al*, 1965

Occasional cases of thrombocytopenic purpura have been reported by several authors among them Larson 1953, Bolton and Dameshek 1956, Freedman *et al* 1956, Schen and Rabinovitz 1958, and Stratford and Tanaka, 1965 Urticaria after quinidine treatment has been reported by Siegal and Horn 1950 and fever of unknown origin in connection with quinidine by Levy, 1922, Sturnick 1942 Stimson and McKusick 1951 Berley and Saland, 1952 Rose *et al* 1953 and Mosbech and Thomsen, 1959

Follow-up

As for follow up investigations in connection with quinidine conversion there is no material described in which there is a long observation time and in which the quinidine maintenance dose is reported Goldman 1951 was of the opinion that after conversion there was a prompt relapse to atrial fibrillation if the quinidine treatment was discontinued Sokolow and Ball, 1956 reported that 85% of the patients who were reexamined had reverted to atrial fibrillation generally within one week without quinidine These reports gave no definite information as to the frequency of maintained sinus rhythm and complications in connection with the administration — or lack thereof — of a maintenance dose of quinidine, cf Table 1a

B DC countershock

For DC countershock conversion the conversion frequency is high throughout 70—97% cf Table 1b

The risk of arterial emboli is low even without anticoagulants according to Lemberg *et al* 1964 and Hurst *et al* 1964

Dangerous ventricular arrhythmias are extremely rare if the vulnerable period of the T wave in the ECG is avoided

There are several short follow-up studies with varying periods of observation after DC countershock conversion No definite conclusions as to maintained sinus rhythm and possible maintenance therapy with quinidine have been made here either Hurst *et al* 1964, converted 117 of 121 patients (97%) After 3—12 months with quinidine sulfate, 0 2 g 4 times daily 102 patients i e 84%, maintained sinus rhythm for varying observation times In 26 patients the quinidine treatment was discontinued because of toxicity symptoms All of these patients promptly reverted to atrial fibrillation

Korsgren *et al* 1965 performed follow up examination in 100 of 107 converted patients and found maintenance of sinus rhythm in 43% It was assumed that an adequate maintenance dose of

Broch and Muller, 1957, examined 20 patients 11 of whom were suffering from mitral stenosis All patients showed a significant mean increase in cardiac output during rest and exercise and an increase in stroke volume at rest, an increased oxygen saturation and a diminished arterio venous O_2-difference in sinus rhythm, as compared with atrial fibrillation The patients with mitral stenosis showed a larger increase in stroke volume at rest and during work than did the rest of the series Seven patients were examined during sinus rhythm after withdrawal of quinidine for several days and showed results identical with the rest of the patients

Varnauskas *et al*, 1959, demonstrated in 3 patients with atrial fibrillation that the heart rate during exercise in the sitting position on a bicycle ergometer rose excessively during the first load step, concomitantly with a drop in stroke volume At higher loads the heart rate remained more or less constant, while the stroke volume rose

Gilbert *et al* 1961 found during tread mill work a lower heart rate with sinus rhythm than with atrial fibrillation Cardiac output was determined in two patients and was found to be 25% higher and the stroke volume 91% higher with sinus rhythm than with atrial fibrillation

Following the introduction of DC countershock 1962 Oram *et al* 1963 published an investigation of the cardiac output in 10 patients Immediately following conversion to sinus rhythm there was no change in cardiac output while there was a rise three to sixteen days later in 7 of 9 patients who had maintained their sinus rhythm

Graettinger *et al*, 1963, reported an unchanged cardiac output at rest and during work in the supine position immediately following conversion However the mean stroke volume increased, and the heart rate dropped significantly

Graettinger *et al*, *1964* found that in 16 patients, suffering from atrial arrhythmias, there was a small but significant increase in cardiac output at rest as well as during work in the supine position after conversion 1—2 hours after awakening from anaesthesia Patients, whose heart rate was decreased by 30 beats per minute or more by conversion increased their cardiac output conspicuously, while the increase was less marked in the other patients, and not significant No change in the oxygen consumption was found while a significant lowering of the arterio-venous O_2-difference was present as well as significant decreases of the heart rate and increases of the stroke volume, at rest as well as during work The mean brachial artery pressure fell in those four patients in whom it was measured

Morris *et al*, 1965 examined 11 patients at rest and 4 patients during work without quinidine, on an average 6 hours after DC countershock conversion and at a stabilized oxygen consumption The cardiac output increased significantly after conversion In 7 patients the increase was about 34%, in one patient there was a decrease in cardiac output and in 3 patients there was no change In all 5 patients, who were examined during work there was an increase in

cardiac output with sinus rhythm the mean increase being 17 % 10 of 11 patients showed a diminished arterio-venous O_2 difference at rest and in all 5 patients tested during work there was a significant decrease

Killip and Baer, 1966, reported from a series of 27 patients 24 hours after DC-countershock conversion of atrial fibrillation to sinus rhythm without quinidine, that the cardiac index rose in patients with mitral valvular disease, concomitant with an increase in stroke volume, an increase in heart rate and a drop in calculated peripheral vascular resistance In 6 patients without valvular disease, no significant increase in cardiac index or in stroke volume was found Twenty-six patients were studied during work and in these identical heart frequencies were found for patients with mitral valvular disease while the patients without valvular disease showed a lower heart rate during sinus rhythm

Rodman *et al*, 1966 described the results of 26 patients, converted from atrial fibrillation to sinus rhythm by quinidine treatment and re examined 5—10 days after conversion while taking a maintenance dose of quinidine In all patients the cardiac index was higher after conversion, the mean increase being 22 per cent The smallest increase was found in 6 patients with arterio sclerotic heart disease and a normal cardiac output The mean heart rate fell by 15% the stroke volume increased by 44% and the arterio-venous O_2-difference dropped In addition 3 patients were examined before immediately after and one week after DC-countershock conversion In all 3 patients the cardiac output had increased after one week of sinus rhythm, in comparison with immediately after conversion The stroke volume increased by 45% with no change in the heart rate To conclude, 19 patients were studied 1, 2 and 3 hours after DC countershock conversion Cardiac output and stroke volume rose gradually, while the heart rate remained constant Six months later during a repeated study, the mean cardiac output in these patients was 10% higher than before conversion

Influence of quinidine on hemodynamics

Ferrer *et al* 1948 administered a single dose of 0 8 g quinidine sulfate orally, and demonstrated a drop in 'arterial blood pressure' without any change in cardiac output

McIntosh and Morris 1966 gave quinidine gluconate orally until a serum quinidine concentration of 3—4 mg/l in 5 healthy and in 8 cardiac patients This did not influence the cardiac output, or the heart rate either at rest or during exercise

Schroder 1966, applying the same technique as we administered quinidine bisulphate Durules® corresponding to 1 6 g quinidine sulphate daily He examined his 6 patients in the reclining position at rest and found no difference in cardiac output stroke volume heart rate, systolic diastolic or mean brachial arterial pressure, and thus also no dif-

ference in calculated peripheral vascular resistance in comparison with the same patient group while receiving placebo. During work there was a significantly lower systolic arterial pressure during quinidine treatment but no other demonstrable differences in the parameters measured.

Present series of patients

Between September 1957 and May 1963 237 patients with atrial fibrillation were subjected to 281 conversion attempts with quinidine at the Cardiac Service of Medical Department I, Sahlgrenska Hospital This was done regardless of the patient s age the cause of the arrhythmia the duration of the fibrillation, the cardiac size, or functional group In all cases, however, the atrial fibrillation had lasted for more than one week prior to the first attempt at conversion The principle was to give all patients with atrial fibrillation at least one chance to restore normal sinus rhythm Only 3 patients who were soon to be operated on for mitral stenosis, 7 patients during I^{131} treatment and 5 special research cases were excluded from the conversion attempts

The patient series comprised 132 patients with valvular lesions predominantly of rheumatic origin They are designated here as rheumatic heart disease, RHD and 56% belonged to this group In addition there were 105 patients without RHD The series was divided because of possible differences in hemodynamics between the groups

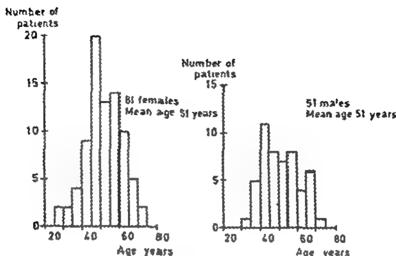
The distribution of age and sex is shown in Fig 1 There was an over-representation of women in the group with RHD and of men in the group without RHD The distribution of different valvular lesions is shown in Table 2a The presence of arterial hypertension in this group was not further investigated

The absence of valvular lesions was determined in all cases on the basis of auscultatory findings and X-rays

The group without RHD comprised patients with coronary disease, hypertension atrial fibrillation without manifest heart disease and 4 patients with treated hyperthyroidism, see Table 2 b Two of the hyperthyroid patients also suffered from hypertension while one suffered from angina pectoris At least 33 patients with hypertension were included in the group Of these 21 had retinal changes of the type fundus hypertonicus I—III according to Keith and Wagener and in addition 12 patients had a blood pressure which exceeded 165/95 mm Hg in the presence of normal sinus rhythm before or after conversion The retinae were not examined in all patients in the group without RHD

Since the blood pressure varies from beat to beat during atrial fibrillation, depending on the diastolic filling time, the number of patients with hypertension that were included in the series is difficult to determine In addition there was no information as to previous blood

RHD



Without RHD

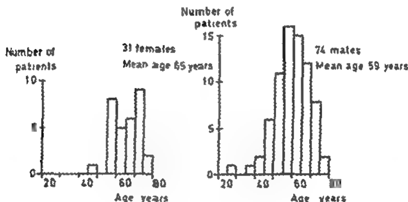


Fig 1 Number of patients sex and age in the groups with and without RHD

pressure during sinus rhythm for some patients who could not be converted The total number of hypertensive patients can therefore not be given in the group without RHD

Statements as to the duration of fibrillation are often unreliable because the patient himself does not know when fibrillation started Some did not notice the arrhythmia others could not differentiate between atrial fibrillation and extra systoles or other arrhythmias

Table 2a. Sex and different valvular lesions of patients with RHD

Diagnosis	No of patients	Female	Male
Mitral stenosis	28	18	10
Operated mitral stenosis	47	30	17
Mitral regurgitation	6	1	5
and stenosis	26	20	6
Aortic valvular lesions	6	3	3
Combined mitral and aortic valvular lesions	19	9	10
Total	132	81	51

Table 2b. Sex and probable diagnosis of patients without RHD

Diagnosis	No of patients	Female	Male
Suspected previous infarction	6	3	3
Coronary heart disease without diagnosed hypertension	13	–	13
Arterial hypertension	33	15	18
Atrial fibrillation without manifest heart disease	37	6	31
Treated hyperthyroidism	4	3	1
Miscellaneous	12	4	8
Total	105	31	74

Table 3. Number of arterial emboli prior to the conversion attempts, their localization and relationship to the diagnosis

Embolus to	Non op mitral valvular lesions	During MS op	Post MS op	Without RHD
Medial cerebral artery	29	1	1 (4 days post op)	11
Posterior inferior cerebellar artery	1	0	0	0
Retinal artery	2	0	0	1
Cochlear	0	1	0	0
Brachial ,,	2	0	0	0
Renal	3	0	0	0
Femoral ,,	14	1	2 (13 days post op 5 years post op)	1
Total 70	51	3	3	13

Often, the atrial fibrillation was detected by chance in connection with a visit to a doctor for some other reason As to the patients in this material all available information on previous ECG and auscultation was obtained In many cases this verified both short and long durations The verifiable duration of fibrillation in these patients varied between 7 days and 16 years

Fifty two of 237 patients, i e 22%, had previously had one or more arterial emboli Of these, 39 patients (32 women and 7 men) with mitral stenosis or operated mitral stenosis had had a total of 57 embolic episodes Thirteen patients without RHD (6 women and 7 men) had had one arterial embolus each In all, there were thus 70 anamnestically and clinically probable or suspect peripheral, arterial embolic episodes, see Table 3

Of a total of 148 converted patients, 144 could be observed for at least 4 years or until they relapsed to atrial fibrillation Two of these patients died during the observation period Two hundred and thirty four patients were observed for at least 28 months with respect to mortality and embolic episodes

Comments

Comparable conversion results of several unselected patient series with chronic atrial fibrillation have been published, but the composition of the series has been somewhat different, e g with respect to diagnosis Thus, Yount *et al*, 1952 described the conversion results of 155 unselected patients of whom only 30% had RHD while Maurice *et al* 1956 treated 313 unselected patients of whom 55% had RHD Freeman and Wexler 1960 described 100 unselected patients of whom only 11% had RHD Rokseth 1963, reported conversion results for 200 entirely unselected patients with a RHD rate of 47% Blondeau *et al* 1960 and Sandoe *et al* 1965 have described a patient series which was probably not selected the former author 575 patients (65 RHD) the latter 100 patients (61% RHD)

These unselected series are comparable to ours The conversion results in particular are comparable while the follow up study differed in several ways from ours Thus, Yount *et al* for instance observed only 41 of 119 converted patients during 1—36 months while Maurice *et al* carried out follow up examinations in only 69 of the 257 converted patients Sandoe *et al*, too made follow up examinations in only 34 of 58 converted patients Blondeau *et al* made follow up studies for as brief a period as 7 days and Freeman and Wexler observed the patients only during their stay in the hospital The follow up investigation which is most comparable to ours is that of Rokseth in which 94 of 107 converted patients were observed for at least 6 months

Other patient series with more than 100 patients have been selected in different ways Hay, 1924 for instance pub-

lished a series (265 patients) compiled from several different clinics which was not treated uniformly Neither the principles of the treatment nor the selection can be assessed by the present author Friedberg and Sjoestroem 1956 (133 patients) excluded subjects who had recently suffered from embolus as well as patients who had extra systoles or different degrees of heart block Sokolow and Ball, 1956, (177 patients) excluded patients with a fibrillation duration exceeding 6 months as well as elderly patients who did well on digitalis alone

As for Kissane *et al*, 1961, (368 patients) it is impossible to evaluate the selection of the patient series Probably, there has been a certain selection as the authors stated that cases included at the end of the investigation were more severe than at the beginning

In these selected series the results of the follow up investigations are not comparable to ours

The treatment during the follow-up period has varied and the literature contains only scattered information on this point

Methods

Clinical and laboratory methods

The patients were examined on a cardiologic ward at the Medical Department. The *diagnosis* was based on the auscultatory findings. For all patients these findings were compiled with the results of heart X-rays according to Jonsell, 1939. In 63 cases these data were supplemented with cardiac catheterization and pressure determinations, in 29 cases with cardioangiography, Kjellberg *et al.*, 1959, and Paulin and Varnauskas, 1962, and in 52 cases with electrokymography according to Bartley, 1960.

The *duration of atrial fibrillation* was determined as accurately as possible by obtaining all available ECG's and records of previous visits to doctors or of hospital confinements. Old ECG's which showed atrial fibrillation gave us definite information as to the minimum duration of atrial fibrillation in many cases, even when the patient was unaware of his arrhythmia. Old ECG's which showed sinus rhythm gave us a limit of the duration of the fibrillation and supplied information as to the possible maximum duration of the atrial fibrillation. In many cases it was impossible to determine the maximum duration. Patients without RHD had more rarely been checked by a doctor prior to the relevant hospital admission than patients with RHD. Therefore, it was more difficult to determine accurately the maximum duration of the fibrillation in patients without RHD.

The patients were classified according to New York Heart Association, 1953, in *functional groups* I—IV. Eight patients were grouped prior to digitalization, and may have been classified too severely according to this. Seven of these patients belonged to Group IV and one to Group III.

During the *conversion attempts* the patient was hospitalized. During the actual quinidine therapy the patient was confined to bed and was strictly controlled clinically. During the quinidine therapy most patients received pentobarbital 0.05 g 3 times daily.

Dicoumarol was given to all patients a few days before and during the quinidine therapy, when a prothrombin index between 40 and 60 units, Lehmann, 1942, was desirable.

When the quinidine therapy was started all patients were digitalized. In 108 cases with cardiac decompensation or arterial hypertension the patients were given chlorothiazide or chlorthalidone

Table 4 Patient series in relation to the treatment method at the first successful or the last unsuccessful conversion attempt

Diagnosis	Sex	Ordinary tablets Quinidine sulphate No of pat.	Quinidine Durules® No of pat	Test dose only No of pat.
RHD	Female	70	9	2
	Male	39	9	3
Without RHD	Female	22	8	1
	Male	56	14	4
Total		187	40	10

and in a few cases mercurial diuretics during the last week before conversion attempt In 135 patients the serum potassium was checked before and twice daily during the quinidine therapy at the same time that the plasma quinidine concentration determination was made Plasma quinidine was determined in 155 patients

The conversion attempts were made partly with ordinary tablets of quinidine sulphate in 187 patients, partly with sustained release tablets (Quinidine Durules® Hassle each containing quinidine bisulphate corresponding to 0 2 g quinidine sulphate) in 40 patients, for various reasons, 10 patients received only a test dose of quinidine, see Table 4

Prior to the quinidine therapy all patients received a test dose of 0.2 g quinidine sulphate and prior to the conversion with Durules® also a larger test dose corresponding to 0 1 g quinidine sulphate/10 kg body weight to test for any possible hypersensitivity

During the quinidine therapy quinidine sulphate was given in the ordinary tablet form usually in a dosage of 0 4 g every other hour and 6 times per day for up to 3 days The dosage was increased gradually during the following days up to 0 5—0 6 g, in occasional cases up to a maximum of 0 7 g 6 times daily, depending upon the individual quinidine tolerance of the patient. Some patients with a known quinidine tolerance started with a higher dose and in 2 patients the initial dose was 0 3 g 6 times daily

Conversion with quinidine Durules® was attempted at a dosage of 0 8 g 2—3 times daily up to 1 6 g 3 times daily with a six hour interval during the day

ECG was taken twice a day once in the morning before the first quinidine dose and once during the day before the quinidine dose at 2 p m. The pulse was taken by a nurse before each quinidine dose

Whether the therapy was to be continued or not was decided individually and was dependent on side effects ECG changes and starting in 1959 also on the quinidine concentration in the plasma Such decisions were made twice a day and always by the same doctor before the 8 a m. and 2 p m quinidine doses

Indications for discontinuing quinidine therapy were vomiting occurrence of a series of ventricular extra systoles pro-

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When the quinidine therapy was started all patients were digitalized. In 108 cases with cardiac decompensation or arterial hypertension the patients were given chlorothiazide or chlorthalidone

them showed symptoms of cardiac decompensation at rest

Hemodynamic investigations were made in the same 24 cases before and after conversion and in an additional 59 patients during atrial fibrillation only. The determinations were made in the morning on patients who had fasted for 12 hours. Examinations were made both at rest with the patient reclining in a chair and during exercise on a bicycle ergometer after ▮ minutes cycling and with a load of 200 and if possible also 400 kpm/min.

Cardiac output determination was made by means of the dye dilution technique with bromsulphalein according to Wassen 1956. The dye was injected into the subclavian vein or, in 45 subjects, into right atrium. Polyethylene catheters were used for the brachial artery and subclavian vein. An X-ray opaque catheter according to Ödman was used for the right atrium. Arterial blood collection from the brachial artery commenced at the time of dye injection, was performed using heparinized Ellerman tubes placed in a motor driven collection device. At rest, each tube sampled arterial blood for 2 seconds and during exercise for 1 second.

Oxygen consumption was determined at the same time as the cardiac output by having the patients inhale room air through a nozzle and exhale into a Douglas bag. The volumes were measured with a gasmeter and the pulmonary ventilation expressed in liters per minute BTPS (37° C, actual pressure and air saturated with water vapour). The oxygen and carbon dioxide were analyzed according to Scholander, 1947. Oxygen consumption was calculated and expressed in *ml* per minute *STPP* (0° C, 760 mm Hg and dry air).

The *intraarterial blood pressure* was registered by means of an Elema strain gauge manometer and a six lead channel electrocardiograph for 10—30 seconds. In a few registrations during exercise periods between 5—10 seconds were used. The mean pressure was obtained by means of electrical integration. Systolic and diastolic pressures were calculated as the mean of the varying pressure peaks on the curve.

The *heart rate* was registered by means of a precordial lead during the determination of cardiac output. The electrodes were placed on the forehead, and at the apex of the heart. An ECG record of 10—30 seconds' duration was used for the calculation.

The hemodynamic investigations, the determinations of cardiac output, of oxygen consumption and of intraarterial blood pressure were performed by the standard methods of the laboratory previously applied by Bojs, 1961, Malmcrona, 1965, Forsberg, 1965 and Sannerstedt, 1966.

Determinations of the *error of the method* for the hemodynamic investigations in atrial fibrillation were made only on the basis of values obtained from patients at rest using the lowest cardiac output in the material cf. Table 5. These cardiac output curves were most difficult to calculate and should give the highest error of the method for cardiac output determination in this series. There were no significant differences between the

nounced widening of the QRS complex exceeding 0 11 seconds, ventricular rate exceeding 110 beats/min flutter for more than 3 consecutive days or a quinidine concentration exceeding 6—7 mg per liter plasma which would increase the risk of toxic side effects Sokolow and Ball, 1956

Nausea dizziness, diarrhea or flutter for 1—3 days did not constitute an indication for discontinuation Side effects in the form of frequent diarrhea were counteracted with tincture of opium

For the *quinidine concentration determination in plasma* a double extraction method with benzene and sulphuric acid and fluorescence determination according to Brodie *et al* 1947 b Udenfriend 1962, Cramer and Isaksson 1963 was used The 24-hour minimum and maximum concentrations were determined by sampling immediately before the morning dose when the two quinidine tablet types were used 2 hours after the evening dose when ordinary quinidine sulphate was used and 3—4 hours after the evening dose when quinidine Durules® were used A concentration of at least 5 mg quinidine per liter plasma was attempted before any conversion attempt was abandoned

When there was a conversion to sinus rhythm the patients were allowed to move about without administration of pentobarbital and the dicoumarol dosage was discontinued gradually As a rule the patients left the hospital a few days later

Most converted patients received a *maintenance* dose of 0 4 g quinidine sulphate 4 times during the day at regular intervals or, after 1962 an equivalent number of quinidine Durules®, 0 8 g twice a day morning and evening Thirty nine patients received Durules® Owing to side effects the size of the dose was subsequently adapted to the individual patient

In 12 patients the maximum concentration of quinidine in the plasma was determined repeatedly 4 hours after the morning dose during maintenance therapy with quinidine Durules®

The *roentgenological cardiac size* during atrial fibrillation prior to the quinidine therapy was determined in all cases by routine X rays while the patients were in a standing position according to Jonsell, 1939 In 13 patients the roentgenological cardiac size in the prone position was determined before and after conversion to sinus rhythm according to Larsson and Kjellberg, 1948 as modified by Kjellberg *et al* 1949

All roentgenological cardiac sizes were checked by one and the same roentgenologist Consideration was given to whether continuous digitalis or diuretic therapy had been started between the date of the X ray and the conversion date (or date of unsuccessful conversion) Such cardiac sizes were excluded from the statistical calculations

ECG during exercise was performed in 26 patients both during atrial fibrillation and sinus rhythm at the Clinical Physiological Laboratory in accordance with their standard procedure Sjostrand 1960 No patients were confined to bed at the time of the two examinations All of them were digitalized and none of

single quinidine dose in the form of ordinary tablets and after Durules®, Cramer *et al* 1963 This investigation showed that there was a strong individual variation for both tablet forms, both with respect to the maximum concentration and the time interval between the administration of the doses and the maximum concentration of quinidine in plasma The median value for the time interval between administration of the dose and the maximum concentration obtained was 1 5 hours after ordinary tablets and 3 9 hours after Durules® After the latter, the concentration remained rather constant for several hours

Throughout we therefore used a 2 hour interval for the administration of the ordinary quinidine sulphate tablets and a 6-hour interval for the Durules®

Since quinidine has a cumulative effect for some days the dose was usually not increased until after 3 days Cramer *et al* 1963

The *quinidine determination method* used has a very small error of a single determination 1 per cent, Cramer and Isaksson 1963 The method involves determination of quinidine alone without quinidine metabolites Brodie *et al* 1947 [b] This method gives decidedly lower values than the often used precipitation method with metaphosphoric acid which includes quinidine metabolites Brodie and Udenfriend, 1943 The method has been used for two reasons When the investigation was made, nothing was known about the antiarrhythmic effect of the quinidine metabolites The fact that they are less effective than quinidine was reported by Conn in 1964 Secondly we wanted to be able to give high quinidine doses at short intervals and thus follow the conversion and risk concentrations which were reported by Sokolow and Ball in 1956 Therefore, it was most practical to use an extraction method for the determination of the quinidine concentration which as much as possible agreed with theirs The differing extraction agents are due to the fact that Brodie *et al* 1947[a] reported that benzene extracts a smaller amount of metabolites than ethylene dichloride The latter solvent was used by Sokolow and Edgar 1950 and Edgar and Sokolow, 1950

Few authors have measured the quinidine concentration in the plasma during the conversion itself Yount *et al*, 1952, Sokolow and Ball 1956 and Hamfelt and Malers 1963 The literature contains no information on the quinidine concentration during the follow up period except for the publication of Engstrom 1967

Statistical methods

Means standard deviations and standard errors were calculated as described in current textbooks on statistics Snedecor 1963

Differences between two means were analyzed using Student s t test and differences between distributions were examined by χ^2 analysis using Yates correction factor The changes within the patients were determined using

t tests based on differences between paired observations The standard error of a single determination was calculated according to Dahlberg 1948

The calculations of relative number of patients in maintained sinus rhythm was carried out according to the technique of calculating survival rates, Herdan 1955

Test for linearity was performed according to Hyrenius, 1962

Most calculations were performed with aid of an electronic desk computor Olivetti, Programma 101

Results of conversion attempts

The results of the conversion totally, and within the groups with and without RHD are shown in Table 6 Of the 237 patients, 148 could be converted (i e 62%) by 281 conversion attempts (53% successes)

After a *test dose* of 0 2 g quinidine sulphate one patient with a treated hyperthyroidism converted to sinus rhythm Three patients had side effects in the form of nausea and thus no further attempts at conversion were tried One woman with mitral stenosis and combined aortic valvular disease developed a cerebral embolus and renal arterial embolus three days after the test dose and died before quinidine therapy could be commenced

After the larger test dose of 0 1 g quinidine sulphate/10 kg body weight before a planned conversion with Durules® 4 out of 45 patients converted to sinus rhythm. One patient suffered from diarrhea and thus no further quinidine was administrated

The conversion frequencies were analysed with respect to sex age diagnosis duration of atrial fibrillation, roentgenological relative cardiac size functional groups, hemodynamics and concentration of quinidine in plasma

Table 6 Patients distributed according to sex diagnosis RHD or without RHD and the conversion frequencies within the groups

Diagnosis	Sex	Total No of pat.	Restored SR No of pat.	Restored SR Per cent of total
RHD	Female	81	51	63
	Male	[illegible]	23	45
Total		132	74	56
Without RHD	Female	31	26	84
	Male	74	48	65
Total		105	74	70
Grand total		237	148	62

$p < 0.05$ (56 vs 70)

Conversion rate in relation to different factors

Sex

The χ^2 test did not indicate that there was any definite difference between the sexes with respect to the conversion frequencies for the whole patient series, nor was there any difference between the sexes as regards the conversion frequency within the groups with and without RHD, see Table 6.

Age

A χ^2 analysis showed no significant differences in the conversion frequencies between different age groups. This applied both to patients with or without RHD, see Table 7.

Diagnosis

The results of the conversion in relation to the diagnosis are shown in Table 8. The conversion frequency was significantly higher for patients without RHD (70%) than for patients with RHD (56%), $p < 0.05$. Table 6.

Table 7. Conversion results in the different age groups

Diagnosis	Age years	Total No. of pat.	Restored SR No. of pat.	Restored SR Per cent of total
RHD	< 45	34	21	61
	45–59	70	39	56
	60–	28	14	50
Without RHD	< 45	4	2	—
	45–59	42	29	69
	60–	59	43	73

Table 8. Conversion frequency to normal sinus rhythm in the different diagnostic groups

Diagnosis	Total No. of pat.	Restored SR No. of pat.	Restored SR Per cent of total
Mitral stenosis	28	18	64
Operated mitral stenosis	47	30	64
Mitral regurgitation	6	3	—
Mitral regurgitation and stenosis	26	12	46
Aortic valvular lesion	6	3	
Combined mitral and aortic valvular lesions	19	8	42
Coronary heart disease Arterial hypertension Atrial fibrillation without manifest heart disease Miscellaneous	101	70	70
Treated hyperthyroidism	4	4	
Total	237	148	62

The conversion frequency was the same for patients with operated and non operated mitral stenosis.

The χ^2 test showed no significant difference in conversion frequency between the group with mitral stenosis (operated as well as non operated) versus the group with mitral insufficiency or combined mitral valvular lesions.

In 8 cases conversion attempts were made in the same patient both before and within 1 year after operation for mitral stenosis. Prior to the operation 6 patients converted to sinus rhythm but relapsed to atrial fibrillation. After the operation 5 of these patients and one other patient could be converted to sinus rhythm.

Duration of the atrial fibrillation

When a comparison was made between the 25 patients in this series who had a verified duration of less than 6 months and those whose duration exceeded 6 months or 1, 2, 3 and 5 years, a χ^2 test revealed a statistically significant difference in the conversion frequency to normal sinus rhythm, $p < 0.001$ in all groups (see Table 9). Patients with a duration of less than 6 months had a significantly higher conversion frequency. The conversion results do not become progressively worse as the duration of atrial fibrillation increases. Thus when the duration of the fibrillation is shorter than 6 months the conversion frequency is high, about 80% for patients with RHD. If the duration exceeds 6 months the conversion frequency is low, about 40%, regardless of whether or not the fibrillation has lasted for 6 months or for more than 5 years (see Fig. 2).

The series did not include patients

Table 9. Conversion frequency in relation to duration of the atrial fibrillation in patients with RHD, without RHD and total

Diagnosis	Duration	Converted	Not converted	
RHD	AF < 6 months	16	3	$p < 0.01$
	AF ≷ 6 months	34	45	
Without RHD	AF < 6 months	6	0	$p < 0.05$
	AF ≷ 6 months	18	20	
Total	AF < 6 months	22	3	$p < 0.001$
	AF ≷ 6 months	52	65	

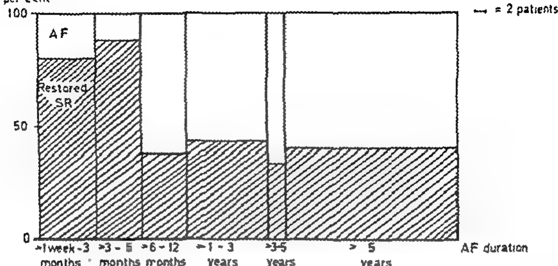


Fig 2. Percentage of conversion to sinus rhythm in relation to the duration of the atrial fibrillation in patients with RHD

without RHD and with a verified duration of atrial fibrillation in the groups > 3—6 months, > 6 months—1 year or > 3—5 years. As for patients without RHD, it is thus possible to say only that cases with a duration of less than 6 months were more easily converted than patients with a duration exceeding 6 months, $p < 0.05$, see Table 9.

A comparison between patients with or without RHD and with a duration of ≤ 6 months showed that there was no significant difference in conversion frequency, see Table 9.

Roentgenological relative cardiac size

In the group with RHD and atrial fibrillation for less than 6 months there was no significant difference in conversion frequency between patients with a relative cardiac size of less than 600 ml and patients with 600 ml or more per m² BSA. Thus the woman who had a combined mitral valvular lesion and who had the largest relative cardiac size among the women, 990 ml per m² BSA, could be converted to sinus rhythm. She had a duration of atrial fibrillation of 3 months.

In patients with RHD and atrial fibrillation for 6 months or more there was a significant difference between various patient groups. Thus the conversion frequency was higher in patients with a relative cardiac size of less than 600 ml than in patients with a cardiac size of 600 ml or more per m² BSA, $p < 0.02$, see Table 10.

Table 10 Conversion frequency in relation to relative cardiac size
Patients with or without RHD (males + females) with duration of the atrial fibrillation ≥ 6 months

Diagnosis	Cardiac size ml/m² BSA	Converted	Not converted	
RHD	< 600	10	3	
				$p < 0.02$
	≥ 600	19	35	
Without RHD	< 600	13	8	
	≥ 600	3	7	

Table 11 Patients distributed according to RHD, without RHD, sex and functional group — converted and not converted

Diagnosis	Sex		Functional group I	II	III	IV	Total
RHD	Female	converted	4	19	23	5	51
		not converted	2	9	17	2	30
	Male	converted	5	7	10	1	23
		not converted	5	14	4	5	[illegible]
Without RHD	Female	converted	3	9	9	5	26
		not converted	1	1	2	1	5
	Male	converted	10	15	14	9	48
		not converted	9	11	6	0	26
Total			39	85	85	28	237
		converted	22	50	56	20	
		not converted	17	35	29	8	

There were too few patients without *RHD* and with a verified duration of less than 6 months in the series to make it possible to test for a difference in the conversion frequency between roentgenological cardiac sizes of less than 600 ml and cardiac sizes exceeding 600 ml per m² BSA.

Among the patients without RHD and with a duration of 6 months or more there was no significant difference in the conversion frequency between relative cardiac sizes of less than 600 ml per m² BSA and sizes of 600 ml or more per m² BSA, see Table 10.

Functional groups

No significant differences were found with respect to functional groups between patients who could be converted to sinus rhythm, neither in the whole series nor in patients with or without RHD, cf. Table 11.

The χ^2 tests were performed between patients in groups I+II and patients in groups III+IV.

Hemodynamics

An investigation of a possible hemodynamic difference was made in a total of 81 patients — 47 who could later be converted to sinus rhythm and 34 who could not

The patients were divided into groups of patients with or without RHD and on the basis of sex see Table 12 a, b, c.

A comparison between the different patient groups at rest and during exercise, using Student's t test, showed that the groups later converted and not converted were comparable with respect to age, height and weight

With respect to cardiac output, heart rate, stroke volume, systolic, diastolic and mean arterial pressure, oxygen consumption and arterio-venous O_2 difference, the t test did not indicate any significant difference between patients who could be converted and patients who could not with three exceptions This applied both while the patients were at rest, reclining in a chair or during work for 8 minutes on a bicycle ergometer with a load of 200 kpm/min

The only significant differences found were for women with RHD who at rest

Table 12 a b c Hemodynamic data during atrial fibrillation in patients who could subsequently be converted and in patients who could not be converted values at rest and during a work load of 200 kpm per minute Figures in parentheses denote number of patients

RHD Females rest AF		later converted			not converted		t test
		mean	SE		mean	SE	
HR beats/min	(19)	69 5	± 2.7	(14)	63 1	± 2.5	
BA_S mm Hg	(19)	145.2	± 5 5	(14)	148 4	± 4 4	
BA_D	(19)	77 8	± 3 2	(14)	79 2	± 3 1	
BA_M	(19)	99 5	± 3 6	(14)	102.1	± 3.3	
CO l min	(19)	3 28	± 0 09	(14)	3 57	± 0 12	
SV ml	(19)	48 7	± 2.4	(14)	57 6	± 2.6	$p < 0.02$
O_2 cons ml/min	(17)	229.2	± 8 0	(11)	230 6	± 5.3	
A V diff ml/l	(17)	70 3	± 2.5	(11)	66.2	± 2.7	
Difference	exercise 200 kpm/min — rest						
		mean	SE		mean	SE	
HR beats min	(9)	+ 72.8	± 9 7	(5)	+ 99 4	± 17 7	
BA_S mm Hg	(9)	+ 27 6	± 6.3	(5)	+ 40 0	± 16 7	
BA_D	(9)	+ 13.2	± 2.5	(5)	+ 21 0	± 7.3	
BA_M	(9)	+ 18 3	± 4 0	(5)	+ 30 0	± 8 7	
CO l/min	(9)	+ 2 52	± 0.31	(5)	+ 3 16	± 0 77	
SV ml	(9)	— 5 9	± 4 4	(5)	— 13 0	± 5 5	
O_2 cons. ml/min	(6)	+434 2	± 64 8	(4)	+599.3	± 3 7	
A V diff ml/l	(6)	+ 52.2	± 9 9	(4)	+ 77.3	± 13 0	

Table 12 b

RHD Males rest AF		later converted			not converted		t test
		mean	SE		mean	SE	
HR beats/min	(13)	70 0	± 5 2	(12)	59 9	± 3 6	
BA_S mm Hg	(11)	125 2	± 3 9	(12)	127 1	± 4 1	
BA_D	(11)	74 1	± 2 4	(12)	71 7	± 3.5	
BA_M	(11)	91 9	± 2 9	(12)	89 8	± 3.5	
CO l/min	(13)	3 91	± 0 20	(12)	3 69	± 0 15	
SV ml	(13)	59 4	± 4 8	(12)	65 1	± 6 1	
O cons ml/min	(10)	247 0	± 9 9	(12)	255 9	± 7 0	
A V diff ml/l	(10)	66 5	± 3 8	(12)	69 9	± 2.3	

Difference exercise 200 kpm/min — rest

		mean	SE		mean	SE	
HR beats/min	(8)	+ 75 9	± 7 3	(6)	+ 62.5	±11 8	
BA_S mm Hg	(6)	+ 26 5	± 10.3	(6)	+ 23 7	± 3 6	
BA_D	(6)	+ 12.7	± 3 4	(6)	+ 12.0	± 4 7	
BA_M	(6)	+ 15 5	± 5 4	(6)	+ 17.3	± 4 8	
CO l/min	(8)	+ 2.58	± 0.25	(6)	+ 3 45	± 0.27	$p < 0.05$
SV ml	(8)	− 13 5	± 4 6	(6)	− 8 2	± 3 2	
O cons ml/min	(7)	+548 1	± 23 4	(6)	+737.3	±91 2	
A V diff ml/l	(7)	*+ 56 0*	*± 4 5*	(6)	*+ 67 2*	± 8 0	

had a higher stroke volume among the 14 non converted patients $p < 0.02$ see Table 12 a

For men with RHD, the cardiac output at a work load of 200 kpm/min was significantly higher in the group that could not be converted than in the group that did convert ($p < 0.05$) see Table 12 b

For men without RHD the stroke volume decreased more during exercise for the group that could not be converted, than in the other group, see Table 12 c

Quinidine concentration

Determination of the quinidine concentration in plasma was made in 155 patients at the first conversion to normal sinus rhythm or at the last unsuccessful conversion attempt in those patients in whom sinus rhythm could not be restored see Tables 13 a and b

As for the other 82 patients in the series no concentration determination was made in 65 patients primarily because the conversion attempt was made before the quinidine determination method was developed (1959) In 17 pati-

Hemodynamics

An investigation of a possible hemodynamic difference was made in a total of 81 patients — 47 who could later be converted to sinus rhythm and 34 who could not

The patients were divided into groups of patients with or without RHD and on the basis of sex see Table 12 a b c

A comparison between the different patient groups at rest and during exercise using Student s t test showed that the groups later converted and not converted were comparable with respect to age height and weight

With respect to cardiac output heart rate stroke volume systolic diastolic and mean arterial pressure oxygen consumption and arterio-venous O difference the t test did not indicate any significant difference between patients who could be converted and patients who could not with three exceptions This applied both while the patients were at rest reclining in a chair or during work for 8 minutes on a bicycle ergometer with a load of 200 kpm/min

The only significant differences found were for women with RHD who at rest

Table 12 a b c H modynam data dur ng a rial fib lat on n pa ient who ould sub quently be convert d and n pa nts who ould not be conve ed value at re t and dur ng a work load of 200 kpm per m nute F gures n parenthe es denote number of pat nt

RHD Females rest AF		la er con erted			not converted		t test
		mean	SE		mean	SE	
HR beats m n	19)	69 5	2 7	(14)	63 1	+ 2.5	
BA_S mm Hg	(19)	145 2	5 5	(14)	148 4	+ 4 4	
BA_D "	(19)	77 8	3 2	(14)	9 2	+ 3 1	
BA_M "	(19	99 5	3 6	(14)	102.1	+ 3.3	
CO l min	(19)	3 28	0 09	(14)	3 57	0 12	
SV ml	(19)	48 7	2.4	(14)	57 6	+ 2.6 p < 0 02	
[illegible] ml/min	17)	229 2	8 0	(11)	230 6	5.3	
A diff ml l	(17)	0 3	2 5	(11)	66.2	2.7	
D [illegible] ence	exercise 200 kpm min — rest						
		mean	SE		mean	SE	
HR bea s min	9	72.8	9 7	(5)	+ 99 4	17 7	
BA mm Hg	9	27 6	6.3	(5)	40 0	16 7	
BA	9	13.2	2 5	(5)	21 0	7 3	
BA_M	[illegible]	18.3	4 0	(5)	+ 30 0	8 7	
CO m n	9	52	0.31	(5)	+ 3 16	+ 0 77	
SV m	9	5 9	4 4	(5)	13 0	5 5	
O ons ml m n	(6	434	64 8	(4)	+599.3	3 7	
A V d f ml l	(6	5	9 9	(4)	+ 77.3	12 0	

Table 13a   Highest quinidine concentration in plasma prior to the first conversion to sinus rhythm

Diagnosis	Sex	No of pat Total	No of pat OT	No of pat D	Maximum concentration of quinidine in plasma mean mg/l Total	OT	D
RHD	Female	26	23	3	5.4	5.4	5.2
	Male	15	13	2	4.6	4.7	3.5
Without RHD	Female	20	13	7	4.4	4.8	3.7
	Male	35	30	5	4.1	4.2	3.4
Total		96	79	17	4.6	4.8	3.9
				range	(1.1-9.6)	(1.2-9.6)	(1.1-6.6)

Table 13b   Highest quinidine concentration in plasma at the last unsuccessful conversion attempt in patients who had not been restored to sinus rhythm

Diagnosis	Sex	No of pat Total	No of pat OT	No of pat D	Maximum concentration of quinidine in plasma mean mg/l Total	OT	D
RHD	Female	18	16	2	5.4	5.5	3.9
	Male	17	13	4	5.3	5.4	5.3
Without RHD	Female	4	4	0	4.2	4.2	—
	Male	20	15	5	4.8	4.8	4.9
Total		59	48	11	5.1	5.1	4.8
				range	(1.2-9.4)	(1.2-9.4)	(3.4-7.0)

OT = Quinidine sulphate in ordinary tablets
D = Durules®

Table 14   Patients distributed according to RHD, without RHD, sex and maximum concentration of quinidine in plasma prior to conversion to sinus rhythm

Diagnosis	Sex	Maximum concentration of quinidine in plasma in converted patients: 0-3 mg/l No	> 3-5 mg/l No	> 5-7 mg/l No	> 7 mg/l No	Total No
Converted with RHD	Female	2	6	15	3	26
	Male	3	4	7	1	15
		15		26		
Converted without RHD	Female	4	9	5	2	20
	Male	10	14	9	2	35
		37		18		
Total		19	33	36	8	96
		20%	34%	38%	8%	

Patients without RHD were converted after a lower maximum concentration of quinidine than patients with RHD $p < 0.01$

Table 15 Patients with attacks of syncope For abbreviations *see p 6*

Case no.	Sex	Age years	Functional group	Diagnosis	Minimum AF duration years	Rtg. cardiac size ml/m² BS	Quinidine			Potassium mEq/l the same day as syncope	Diuretics within the last 2 days before syncope
							total dose g	concentration mg/l plasma			
								max	at attack or after		
1	M	46	II	MS+MI	1 –	790	4.0	3.6	–	–	–
2	F	46	I	AS+LBBB††	2/12	–	2.0	7.3	7.3	4.1	chlorothiazide
3	F	47	III	MS	1 11/12	530	8.4	–	–	–	–
		47	II III	MS op	[illegible]/12	420	4.4	–	–	–	chlorothiazide
4	F	47	II	MS op	3/12	610	3.6	5.3	5.3	–	–
5	F	48	I	MS op	6 6/12	530	3.6	6.2	4.1	3.8	–
6	F	48	II	MS	8 –	730	16.2	> 8.4	–	3.6†	chlorothiazide
7	F	50	II III	MS reop	5/12	690	1.2	–	–	4.1	–
8	F	54	III	MS	3 –	–	2.4	–	–	4.8	–
9	F	54	II III	MS op	11 –	800	5.5	–	–	–	–
10	F	55	I II	MS op	[illegible] 7/12	670	2.4[D]	5.5	5.5	4.0	–
11	F	57	II	MI+AS+AI +RBBB	1/12	620	2.0	–	6.3	4.6	–
12	F	59	II	MS	15 days	640	3.2	4.9	4.3	–	–
13	F	69	I	CHD	23 days	570	4.0[D]	4.4	–	2.9	chlorothiazide
14	F	70	III	HHD	1 7/12	610	12.5	9.0	6.5	6.0	–
15	F	74	II III	HHD†††	2/12	420	2.8	–	–	5.1	chlorothiazide
16	F	74	II	CHD	12 days	750	4.2[D]	5.4	3.8	4.5	chlorothiazide

M male † = 2 days prior to syncope
F female †† = Hemoglobin 8.7 g/100 ml
D Durules® ††† Hemoglobin 9.6 g/100 ml

		Symptoms	1 CG immediately after attack	Conversion to SR	Duration SR
ŋldigitoxin	0.2×1	fainted when getting out of bed	***SR, bradycardia	+	17 days
ŋldigitoxin	0.2×3	0 pulse, 0 heart sounds	*AF	–	–
rum digitalis	50×2	0 pulse, reduced hearing	***Idioventricular rhythm	–	2 days
itoxin	0 1×1 2	0 pulse, vomiting	–	–	–
itoxin	0 1×2	0 pulse 0 heart sounds cyanosis cramps	AF	+	5 months
itoxin	0 1×1	0 pulse, 0 heart sounds cyanosis, amaurosis	***A V dissociation, nodal rhythm	–	1 day
itoxin	0 1×1 2	0 pulse, 0 heart sounds	A flutter 2 1 3 1 block	+	1 day
itoxin	0 1×2	buzzing in ear pale fainted on bed pan	***SR	–	2 months
itoxin	0 1×1	unconscious motoric unrest bite in lip	–	–	–
itoxin	0 1×1	unconscious cyanosis	A flutter 2 1 block, RBBB	–	–
itoxin	0 1×1	unconscious no respiration	***Nodal rhythm	+	2 months [illegible] day
itoxin	0 1×1	unconscious yellowish pale about 10 similar attacks	A flutter	–	–
gitoxin	0 1×1	unconscious, 0 pulse cyanosis, cramps	***SR multifocal VES	+	8 days
etyldigitoxin	0 1×1	unconscious 0 heart sounds	Nodal rhythm, VES	+	1 day
gitoxin	0 1×1	attacks of syncope	**Nodal rhythm	–	5 years 2 month
gitoxin	0 1×1	unconscious	AF	+	1 day
gitoxin	0 1×1	unconscious 2–3 min.	Nodal rhythm VES	+	2 days

* = V fibrillation during attack
** = V tachycardia during attack
** = SR prior to syncope

Table 16 Sex distribution of the patients with syncope compared to the other patients

	Female	Male	Total	
Patients with syncope	15	1	16	$p < 0.001$
Other patients	97	124	221	
	112	125	237	

point in time of the conversion and the quinidine concentration at the time of the conversion

Serum potassium

Serum potassium was measured at the same time as the determination of quinidine concentration in 135 patients. In 75 patients there was a potassium value taken on the same day that the patient converted to sinus rhythm and in 60 patients there was a potassium value on the final day that quinidine was given in an unsuccessful attempt to break the atrial fibrillation. Student's t test revealed no significant difference between these two groups. Both had a mean serum potassium level of 4.3 mg/l, with a SE of ± 0.1.

ECG changes

As for ECG changes during the quinidine therapy *atrial flutter* was recorded in 102 patients, i.e. in 43% of the patient series. Fifty five of these patients could be converted to normal sinus rhythm.

The series included 8 patients with *bundle branch block*, 5 men without RHD and 3 women with RHD. Five of these, 4 men and 1 woman could be converted without complications. Two women with aortic stenosis and bundle branch block had an attack of syncope and did not convert to sinus rhythm.

Complications and side effects

These calculations were made on 377 conversion attempts, that is including all the quinidine reconversion attempts in the patient series.

Death or arterial emboli

No deaths or arterial emboli occurred during the quinidine therapy in direct connection with conversion to sinus rhythm or during the first 24 hours after the establishment of sinus rhythm. This was valid also for those patients who were not converted to sinus rhythm.

Syncope

Unconsciousness occurred 17 times in 16 patients, i.e. in 6.8% of the patients and in 4.5% of the conversion attempts. In 11 cases this took place during quinidine therapy and in 6 cases during the first 24 hours after conversion to sinus rhythm with quinidine as maintenance therapy, see Table 15.

The series of patients with syncope comprised 15 women and 1 man There was a significantly higher incidence of syncope in women than in men in comparison with the distribution between women and men in the rest of the patient series, $p < 0.001$, see Table 16 The incidence of syncope in the whole series was 13 4% for women and 0 8% for men

The mean age of the women with RHD and syncope was 51 years and was equivalent to the mean age for women with RHD but without syncope The mean age for the women without RHD but with syncope was 72 years A t-test showed that the mean age was significantly higher for this group than for the corresponding group without syncope (64 years) $p < 0.05$

RHD was present in 12 patients of whom 2 had aortic valvular lesions with bundle branch block The incidence of syncope was not significantly higher in women with RHD than in women without RHD in comparison to the distribution of women in these two groups in the rest of the series, $\chi^2 = 0.046$

Eight patients had had verified atrial fibrillation for more than 6 months none had had atrial fibrillation for a certainty less than 6 months The minimum duration of the fibrillation is shown in Table 15 There was no significant difference in the duration of the atrial fibrillation between patients with fibrillation for more than 6 months and those with fibrillation for less than 6 months in comparison with the rest of the series

The majority of the patients (9) belonged to functional groups I II The relative cardiac sizes varied between 420 and 800 ml/m² BSA, the mean size was 630 ml/m² BSA

A study of the distribution of women with more and less than 600 ml per m² BSA showed that there was no significant difference in relative cardiac size between women with or without syncope

The mean maximum concentration of quinidine in plasma for the patients with syncope was 6 4 mg/l plasma for women with RHD and 6 3 for women without RHD A t test showed that this concentration did not differ significantly from the concentration found in corresponding groups without syncope

Of the patients with syncope 3 women without RHD had been treated with thiazide and one had a serum potassium value of 2 9 mEq/l, that is below the normal value of 3 6 Of the women without RHD and without syncope 4 of 12 thiazide treated patients had a serum potassium value of less than 3 6 (range 3 1—3 4 mEq/l) The frequency of syncope in the women without RHD and with a serum potassium value of less than 3 6 mEq/l was not higher than in the women with a serum potassium value of 3 6 mEq/l or more

In 11 cases the syncopal attack came unexpectedly without previous indication in the ECG in the form of an increased number of ventricular extra systoles widening of the QRS to more than 0 11 seconds or other arrhythmia except atrial fibrillation or flutter or tachycardia In 6 cases there were such ECG changes During treatment with quinidine there was an increased num

ber of ventricular extra systoles in 3 cases One of these was combined with tachycardia and left bundle branch block, one was combined with tachycardia and 2:1 flutter and one with a widening of the QRS up to 0.14 Moreover, one patient had a regular atrial rhythm with a retrograde conduction and one a nodal rhythm at a high quinidine concentration Of the 6 patients who had already converted to sinus rhythm, one had ventricular extra systoles and nodal extra systoles

There was no higher frequency of ventricular extra systoles during conversion attempts in women with syncope than in those without syncope

Of these 17 patients, 12 converted to sinus rhythm after the syncopal attack All patients were saved, 3 without special treatment and the others by striking the chest or by transthoracic cardiac massage in combination with positive pressure respiration In 3 cases, an intracardiac injection of epinephrine was also given Two patients were given potassium intravenously and one woman with ventricular tachycardia was defibrillated electrically several times In one patient there was a fracture of the sternum as a result of transthoracic cardiac massage

The cardiac rhythm was recorded during the attack in 2 patients and immediately after the attack in 15 patients, see Table 15 The table also shows the relationship to the diagnosis, duration of the fibrillation, roentgenological relative cardiac size, the dose of quinidine administered and the quinidine concentration in plasma, digitalis and diuretic therapy

Table 17 › Main reason for discontinuing the quinidine therapy in the 89 patients in whom sinus rhythm could not be restored

	No of pat
Occurrence of ventricular extra systoles	17
Atrial flutter	18
QRS duration increase > 0.11 sec.	7
Ventricular rate > 110 beats/min nodal rhythm	9
Gastrointestinal side effects	10
Syncope	4
Fever	2
Quinidine concentration > 7 mg/l plasma	7
> 5 mg/l plasma	7
Daily quinidine dose 0.6 g × 6 without effect	2
Thrombocytopenia < 100 000 platelets per mm³	1
Precordial pain or pain in left arm	3
Died 3 days after the test dose before the quinidine therapy had been begun	1
Other	2
	89

Gastrointestinal side effects

Gastrointestinal side effects in the form of nausea, vomiting diarrhea or combination of these were found in at least one of all conversion attempts in 90 of 237 patients, i e in 38% of the patient series Fifty six of these patients however, could be converted to normal sinus rhythm with quinidine in spite of the side effects

The main reason for discontinuing the quinidine therapy in the 89 patients in whom sinus rhythm was not restored is shown in Table 17

Comments

Sex

The conversion results analyzed in relation to sex are not included in the comparable unselected patient series Viko *et al* 1923 considered that women were more easily converted than men Friedberg and Sjoestroem 1956, reported a conversion frequency which was twice as high for men as for women in a selected series The latter finding is unexplainable also to the authors themselves in spite of a careful analysis The finding was not due to a higher frequency of RHD among the women in this material

Our series shows that there was no significant difference between the sexes with respect to the conversion frequency and thus does not support any of the information given in literature

Age

The age of the patient is of no importance to the conversion results in our series This is in complete agreement with Rokseth 1963, and also with *Yount et al*, 1952 Both authors, just as we had patients above the age of 70 in their series As for other unselected series this question is not discussed The fact that age is of no importance to the conversion results has been shown previously several times in small series by among others Frey 1921 Hewlett and Sweeney 1921, Viko *et al* 1923

Diagnosis

A comparison between our series and other unselected patient series of a similar magnitude shows that our total conversion frequency is about the same as that reported previously Table 18

Blondeau *et al* reported a conversion frequency of 62% for the number of conversion attempts which thus should be compared with our corresponding figure for the conversion attempts i e 53%

In our series the conversion frequency is significantly lower for patients with RHD (56%) than for patients with out RHD (70%) This corresponds to other unselected patient series Sandoe *et al* for instance had a conversion frequency of 44% for patients with RHD and 80% for patients without RHD Just as we did they had a relatively large number of patients with mitral stenosis in their series Rokseth too had a lower conversion frequency for patients with RHD than for patients without RHD

Table 18 *Comparison between the present patient series and other unselected patient series comprising at least 100 patients*

	No of patients	Conversion frequency for the whole series %	Composition of the series RHD %	MS %
Yount *et al* 1952	155	76	30	not stated
Maurice *et al* 1956	313	66	55	45
Freeman and Wexler 1960	100	57	11	not stated
Rokseth 1963	200	54	47	20
Sandoe *et al* 1965	100	58	61	61
Own series	237	62	56	43

although the difference was not very great, 48 and 59% respectively Maurice *et al* had a conversion frequency of 58% for patients with RHD which is in good agreement with our conversion frequency Both series contained almost the same relative number of patients with mitral stenosis

Maurice *et al* did not give any information as to the total conversion frequency for patients with non rheumatic heart disease

Yount *et al* reported a higher conversion frequency totally than all other authors This can perhaps be explained by the fact that this material contained a relatively lower number of patients with RHD than our series and other unselected series

Yount *et al* furthermore, reported that the conversion frequency after exclusion of the patients who were considered to have received "incomplete treatment" was 90% for patients with RHD and 85%, for patients with arteriosclerotic heart disease This finding is difficult to evaluate, however He did not state what was meant by "incomplete treatment" which is very important when the composition of the patient series is changed in this manner According to our experience, the post evaluation of the optimal treatment dose is practically impossible in the individual case

Duration of the atrial fibrillation

The duration of fibrillation is of decisive importance to the conversion results In this respect, our series corresponds well with that of Rokseth, 1963 In his series, atrial fibrillation of less than 6 months' duration was converted in 70% of the cases between 6 and 24 months in 44% and more than 24 months in 30% of the cases Maurice *et al* 1956, also reported similar figures fibrillation 7 days–1 month = 78% conversion 1 month–6 months = 59% 1–5 years = 55% more than 5 years = 29%

Sokolow and Ball 1956, showed in a selected patient series, that there was a

considerable drop in conversion frequency with a duration of atrial fibrillation greater than 6 months This was valid for patients with or without RHD

In our series, the patients with RHD could be divided into distinct groups with respect to the duration of fibrillation. Patients with a duration of fibrillation of less than 6 months were converted in about 80% of the cases Sokolow and Ball 1956 found a conversion frequency of 83% for this group If the duration exceeded 6 months the conversion frequency in the present series remained unchanged at about 40% This applied to all groups even if the duration exceeded 5 years

Yount *et al* 1952, on the other hand found no relationship between the duration of fibrillation and the conversion frequency This could possibly be explained by the relatively low number of patients with RHD in his material

Roentgenological relative cardiac size

In patients with RHD and a duration of fibrillation equal to or exceeding 6 months there was an inverse relationship between roentgenological relative cardiac size and the conversion frequency There was a higher conversion frequency in patients with hearts < 600 ml/m² BSA than in patients with hearts $\geqq 600$ ml/m² BSA Table 10 When there was a short duration of fibrillation in patients with RHD this relationship did not exist In this group also very large sized hearts could be converted This indicates that the duration of fibrillation and roentgenological relative cardiac size are factors which independent of one another affect the conversion results

In patients without RHD there was no relation between conversion frequency and roentgenological relative cardiac size when the duration of fibrillation was equal to or greater than 6 months When the duration of fibrillation was shorter, there were not enough cases available to test patients without RHD

The literature gives varying information as to the significance of the cardiac size for the conversion frequency Freeman and Wexler, 1960 reported that 3 patients with the largest cardiac sizes in their unselected series of 100 patients were converted and that, for patients without RHD there was an inverse relation between cardiac size and conversion frequency Sandoe *et al* 1965 maintained that hearts smaller than 500 ml/m² BSA were more easily converted than hearts larger than 700 ml/m² BSA The difference, however was not statistically significant Rokseth 1963 reported *a tendency towards an inverse relation* between cardiac size and conversion frequency in his whole patient series Neither one of the above mentioned authors took into consideration the factor of the duration of fibrillation in making their calculations

Functional groups

In our series there was no difference in the conversion results between functional groups I+II compared with III+IV Similarly Yount *et al* 1952, were not able to prove that there was any relation between functional group and conversion frequency This is in con

trast to Rokseth 1963, who showed a decreasing conversion frequency with increasing functional group

This difference could possibly be explained by the fact that the division into functional groups is relatively approximate and subjective Furthermore the degree of digitalization and dehydration may have differed in the series, which is also difficult to determine

Hemodynamics

Considering patients with atrial fibrillation and at rest, the only difference that could be found between those patients who later converted to sinus rhythm and those who did not, was that the women with RHD who could not be converted had a higher stroke volume than those who could be converted This could possibly be due to a difference in digitalization between the two groups since the heart rate was lower — although not significantly so — in the latter group (average 70 and 63 beats per minute)

Generally speaking, the 34 patients who could not be converted were thus not hemodynamically worse at rest than those who could be converted

The only differences which were observed during exercise were that men with RHD who could not be converted had a somewhat higher cardiac output and that men without RHD had a greater decrease of stroke volume in the not converted group than the patients who could be converted The reaction to exercise was thus not unique in all groups for those patients who could not be converted

The determination of hemodynamics at rest or during exercise is thus of no help when selecting patients for conversion

Quinidine concentration

For the patients in this series, who were converted to sinus rhythm, the maximum concentration of quinidine in the plasma before conversion to sinus rhythm averaged 4 6 mg/l with a range of 1 1—9 6 mg/l This is lower than the corresponding figures reported by Sokolow and Ball, 1956, which is the only series published in which an extraction method was used These authors reported 6 1 mg/l as mean with a range of 1—15 mg/l

The average for the non-converted patients in our series was 5 1 mg/l plasma with a range of 1 2—9 4 mg/l

Thus, the quinidine concentration was not lower in the non-converted group The corresponding figures reported by Sokolow and Ball were 2—19 mg/l

These differences cannot be due to methodological factors, since the quinidine determinations were done by an extraction method in both groups On testing, both methods gave similar values despite the fact that different extraction agents were used The method used by Sokolow and Ball gave only insignificantly higher values, Cramér and Isaksson, 1963

The higher concentrations in plasma indicate that the quinidine administration was higher in Sokolow and Ball's series Their conversion frequency was also higher totally, 86%, as compared

with 62 % in our series It should be noticed however, that their series was selected to include patients with a brief duration of fibrillation This agrees well with our conversion results for patients with a duration of fibrillation of less than 6 months

In our series the conversion to sinus rhythm took place during the night in 68% of the cases The corresponding figure for Sokolow and Ball, 1956 was 15% Apparently, the quinidine con centration in the plasma was decreasing at that time Information is still incomplete as to the condition and activity of the quinidine metabolites

Flutter

Of 148 converted patients in this series, 55 (37%) converted to sinus rhythm via recorded atrial flutter In 18 of 89 patients (20%) the quinidine was discontinued owing to flutter because we interpreted this as a sign of poor digitalization These patients reverted to atrial fibrillation

In the series of Yount *et al* 1952 8 of 119 converted patients went through a stage of atrial flutter before conversion to sinus rhythm Yount *et al* pointed out that the figure probably would have been higher if additional ECG s had been taken They give no information as to how often ECG s were recorded

Other unselected large series do not take up the question of flutter during conversion

Goldman 1951 reported that 26 of 40 converted patients (65%) had converted to sinus rhythm via flutter In an additional 8 patients the flutter remained The total number of non converted patients in the material was not stated As was the case with our series, the patients were controlled by means of ECG twice daily

The difference between our 37% of patients who converted via flutter and Goldman's 65% could possibly have been caused by the fact that Goldman continued with the quinidine treatment up to 7 days of flutter while we discontinued the quinidine administration after 3 days of flutter Perhaps most patients convert to sinus rhythm via flutter, Friedberg 1966

Bundle branch block

According to Di Palma 1950 a bundle branch block would be a strong contraindication for conversion of atrial fibrillation with quinidine This is partly contradicted by our results as 4 of 5 men with bundle branch block and without RHD and 1 of 3 women with bundle branch block and RHD could be converted The other 2 women had aortic stenosis suffered an attack of syncope during the quinidine therapy and did not convert to sinus rhythm

The possibility of converting patients with bundle branch block agrees with the findings of Rokseth, 1963 who con verted 7 of 13 patients without complications Freeman and Wexler, 1960, converted 3 of 5 patients with bundle branch block in their series As for other unselected large series no results with respect to bundle branch block patients appear to have been given

Complications and side effects

Arterial emboli

Using a brief period of treatment with dicoumarol before and during the quinidine therapy, there were no emboli in a total of 377 conversion attempts in this patient series The only episode was a cerebral embolus 2 days after a test dose of quinidine, before the quinidine therapy was started This occurred in spite of dicoumarol treatment and a prothrombin index of 42 units

Neither Freeman and Wexler, 1960, Rokseth, 1963, nor Sandoe *et al*, 1965 had any peripheral arterial emboli in their series during dicoumarol treatment of all patients before and during the conversion attempts In Rokseth's series there may possibly have been a lung embolus

Blondeau *et al*, 1960, had 5 peripheral emboli during 160 conversion attempts without anticoagulants (3 1%) and one embolus during 530 conversion attempts with anticoagulants (0 2%) They used partly heparin partly several prothrombin reducing agents which were not specified Maurice *et al* 1956, had 5 peripheral emboli in 160 patients without anticoagulants (3 1%) and one embolus in 230 patients with different anticoagulants (0 4%) Sokolow and Ball, 1956, had 2 peripheral emboli during conversion attempts in 177 selected patients (1 1%) who with a few exceptions had not received anticoagulants Anticoagulants were given only to a few patients who had recently had a peripheral embolus

Thus, several large series have previously shown that the risk of emboli during conversion is small and is further reduced when the patients are treated with anticoagulants Our series shows that the risk is practically non-existent both in patients with and in patients without RHD provided there is even a moderate dosage with anticoagulants

Syncope and mortality

Syncope with or without apnea occurred 17 times in 16 of 237 patients in our series, i e in 6 8% of the patients In Rokseth and Storstein's series, 1963, there was syncope in 12 of 274 patients, i e in 4 4% of the patients In both series all patients survived thanks to careful observation and fast treatment In Sandoe's *et al* series, 1965, there were 2 cases of syncope in 100 patients but no patient died In the series of Blondeau *et al*, 1960, there were 7 cases of syncope and, in addition to this 7 deaths in 690 conversion attempts Maurice *et al*, 1956, reported 5 cases of syncope and, in addition to this 7 deaths in 313 patients, i e 1 6% and 2 2% respectively In the series of Yount *et al* 1952, there were no incidents of syncope but one sudden death in a series of 155 patients, i e 0 6%

The series which is most comparable to ours is that of Rokseth and Storstein both because of the frequency of syncope and because no patient died Common to both series is the fact that the syncopal attacks most often came without previous warning and also after small doses of quinidine In our syncope material

there was a significant over representation of women compared to the sex distribution of the series as a whole Both of Sandoe's *et al* patients who had syncope were women In the series of Rokseth and Storstein the sex distribution was 9 women and 3 men

Neither Maurice *et al* nor Blondeau *et al* give any information on the sex distribution In Rokseth and Storstein's series, most of the patients with syncope had had fibrillation for more than 2 years and had large hearts All of them had cardiac decompensation and belonged to functional groups III or IV This does not correspond to our series in which most of the patients belonged to functional groups I and II and in which the duration of fibrillation and the cardiac size were not significantly different than that of the women in the rest of the series

Maurice *et al* and Blondeau *et al* reported that cardiac decompensation and large hearts were characteristic of the patients who had a syncopal attack or who died suddenly

In the series of Maurice *et al* 3 of 5 syncopal attacks and 5 of 7 deaths occurred after conversion to sinus rhythm Three of the deaths occurred within 24 hours of the conversion As for the syncopal attacks during sinus rhythm, the time interval between the conversion and the attacks was not stated In Rokseth s series 2 syncopal attacks occurred after transient period of sinus rhythm This can be compared with 6 syncopal attacks within 24 hours after conversion to sinus rhythm in our series

All experience indicates that it is important to observe carefully the patients during quinidine treatment for conversion and when needed, quickly resort to artificial respiration and external cardiac massage. Women are more prone to syncopal attacks during the quinidine administration than men As the syncopal attack may occur also after a small dose of quinidine, the patient should be carefully observed when testing for quinidine sensitivity

Gastro-intestinal side effects

Nausea vomiting or diarrhea occurred during at least one conversion attempt in 90 of our 237 patients However, this did not lead to discontinuation of the quinidine treatment in more than 10 patients who could not be converted The incidence of these side effects varies in different series Maurice *et al*, 1956, reported that 18 of 313 patients had gastro-intestinal side effects as a result of which 9 patients had to discontinue the quinidine treatment In Rokseth's series, 1963, there were gastro-intestinal side effects in 60 of 200 patients

Fever

In 2 cases (0 8% of the patients) fever, 38°C without diagnosed infection caused discontinuation of the quinidine treatment None of the large, unselected patient series give information as to the number of cases with this side effect Occasional cases of drug fever during quinidine treatment have been reported by among others Rose *et al* 1953 No

information as to the frequency seems to have been published

Trombocytopenia

Less than 100000 platelets per mm³, without purpura caused the discontinuation of the quinidine treatment in 2 cases

Several cases of drug induced thrombocytopenic purpura during quinidine treatment have been reported previously, e g in a review by Bolton and Dameshek, 1956, and by Stratford and Tanaka, 1965 In Rokseth's series, 1963, there was 1 case of thrombocytopenic purpura in 200 patients

Follow-up study

Results

Of a total of 148 converted patients 144 could be observed for at least 4 years or until they relapsed to atrial fibrillation Two patients died during observation

The number of patients with maintained sinus rhythm expressed in per cent in relation to the length of the follow-up period is shown in Figs 3 and 4

The curves declined sharply during the first month after which they leveled out This meant that a great number of patients relapsed to atrial fibrillation shortly after the conversion varying between a few hours and up to several weeks

Table 19 shows the number of patients with maintained sinus rhythm and the corresponding per cent of the original group that stayed in sinus rhythm in relation to the follow up period, within the groups with or without RHD, and in women and men Of 148 converted patients, 86 (58%) had sinus rhythm for one month or more while 27 patients (20%) maintained sinus rhythm for 4 years or more

Relapse to atrial fibrillation shortly after the conversion was characteristic

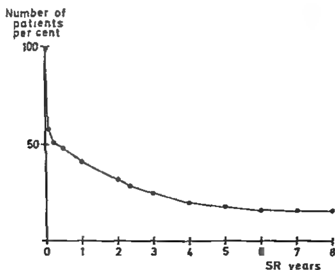
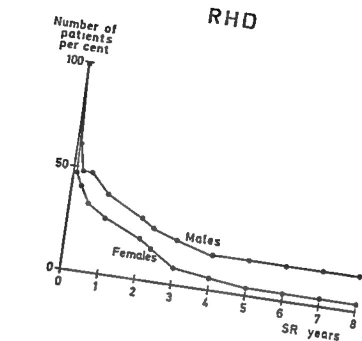


Fig 3 Per cent of patients in sinus rhythm in relation to the duration of maintained sinus rhythm



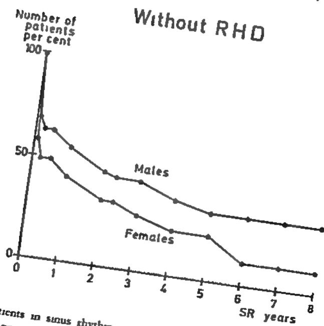


Fig 4 Per cent of patients in sinus rhythm in relation to the duration of maintained sinus rhythm grouped according to diagnosis and sex

Table 19 Patients with maintained sinus rhythm according to the length of maintained sinus rhythm sex RHD and without RHD The percentage figures indicate the number of patients in sinus rhythm in relation to the number of observations Figures in parentheses denote the number of patients excluded for reasons other than relapse to arrhythmia

SR duration	RHD Female %	No	Male %	No	Without RHD Female %	No	Male %	No	Total %	No
		51		23		26		48		148
1 month	47	24	61	14	58	15	69	33	58	86
3 months	41	21	48	11	49	12 (1)	63	30	51	74 (1)
6 months	33	17	48	11	49	12	63	30	48	70
1 year	27	14	39	9	41	9 (1)	56	25 (2)	41	57 (3)
2 years	20	10	30	7	32	7	46	20 (1)	32	44 (1)
28 months	16	8	26	6	32	7	44	19	29	40
3 years	8	4	22	5	27	6	44	18 (1)	25	33 (1)
4 years	6	3	17	4	22	5	37	15	20	27
5 years	4	2	17	3 (1)	22	3 (2)	33	9 (5)	18	17 (8)
6 years	4	1 (1)	17	1 (2)	11	1 (1)	33	5 (4)	16	8 (8)
7 years	4	1	17	0 (1)	11	1	33	3 (2)	16	5 (3)
8 years	4	1	17	0	11	0 (1)	33	1 (2)	16	2 (3)

for all groups but, in this series it was most pronounced for women with RHD and least pronounced for men without RHD Of 51 women with RHD who were converted 47% had sinus rhythm for one month or more compared with 69% in men without RHD

In order to analyze the factors of importance in connection with early relapse to atrial fibrillation, a comparison was made between patients who maintained sinus rhythm for less than 1 month and patients who stayed in sinus rhythm for 1 month or more The reason for this was that in all groups, the highest percentage of relapse to arrhythmia occurred during the first month

Duration of maintained sinus rhythm in relation to different factors

Sex

Comparison of the frequencies of maintained sinus rhythm for less than 1 month and at least 1 month with respect to the sex of the patient, revealed that there was no significant difference between men and women neither in the whole series nor within groups with and without RHD, see Table 20

After 4 years' observation there was a significantly better maintenance of sinus rhythm in men than in women $p < 0.02$ by χ^2 test See Fig 4 and Table 19

Age

There was no difference in age distribution between patients with maintained sinus rhythm for 1 month and patients with *maintained* sinus rhythm for a shorter period of time Regarding patients with RHD, there was no difference between those less than 50 years old as compared to those 50 years or older

Table 20 Patients distributed according to length of sinus rhythm RHD without RHD and sex

Diagnosis	Sex	SR < 1 month	SR ≥ 1 month	Total
RHD	Female	27	24	51
	Male	9	14	23
Total		36	38	74
Without RHD	Female	11	15	26
	Male	15	33	48
Total		26	48	74
Total	Female	38	39	77
	Male	24	47	71
Grand total		62	86	148

Table 21 Patients distributed according to length of sinus rhythm RHD without RHD and age

Diagnosis	Sex	Age years	SR < 1 month	SR ≥ 1 month	Total
RHD	Female+Male	< 50	15	21	36
	Female+Male	≥ 50	21	17	38
Total			36	38	74
Without RHD	Female+Male	< 60	11	20	31
	Female+Male	≥ 60	15	28	43
Total			26	48	74

This was also true for patients without RHD when comparison was made between those less than 60 years of age and those who were 60 or older These age limits were chosen on the basis of the mean age within the groups in question, see Fig 1 and Table 21

Diagnosis

There were more patients without RHD in the group having sinus rhythm for 1 month or more than patients with RHD, but the difference was not statistically significant as shown by a χ^2 test see Table 20

Mitral stenosis Operated and not operated

Comparison of the 31 patients with operated mitral stenosis with 18 not operated patients with mitral stenosis without insufficiency of which 2 were

Table 22 : Patients distributed according to length of sinus rhythm non-operated or operated mitral stenosis and sex

Sex	Diagnosis	SR < 1 month	SR ≥ 1 month	Total	
Female	MS	9	2	11	$p < 0.05$
	MS op	9	14	23	
Total.		18	16	34	
Male	MS	3	4	7	
	MS op	3	5	8	
Total		6	9	15	

combined with aortic disease, the χ^2 test showed no difference in distribution between the number of patients who maintained sinus rhythm for less than 1 month and those who maintained sinus rhythm for 1 month or more

Twenty-three women with operated and 11 with not operated mitral stenosis were included in the series In these patients there was a significant difference between those who retained sinus rhythm for less than 1 month and those who maintained it for 1 month or more $p < 0.05$, see Table 22 The operated patients showed better maintenance of sinus rhythm

Eight men with operated mitral stenosis and 7 with not operated mitral stenosis were included in the series In these patients there was no such corresponding significant difference

Duration of the atrial fibrillation

No patient in the whole material with RHD and atrial fibrillation for 3 years maintained sinus rhythm for 1 month

A comparison between all 29 women and 14 men with RHD and verified atrial fibrillation for less than 3 years and 13 women and 3 men with atrial fibrillation for 3 years or more showed that there was a clear difference in distribution between the number of patients with RHD who maintained sinus rhythm for less than 1 month and those who maintained it for 1 month or more $p < 0.001$, see Table 23 Patients with atrial fibrillation less than 3 years showed better maintenance of sinus rhythm

On the average the group with RHD and atrial fibrillation for 3 years or more stayed in sinus rhythm for 5 days, range 1—27 days Considering the possible maximum sinus rhythm duration before arrhythmia was again established, the group could at the most have had sinus rhythm for an average of [illegible] days, range 1—33 days The maintenance dose of quinidine sulphate in these 16 patients was ≥ 1.2 g per day in 9 cases and < 1.2 g per day in 4 cases in 3 cases the quinidine was discontinued during the first month

Table 23 Patients distributed according to length of sinus rhythm RHD without RHD sex and duration of atrial fibrillation 3 years and ≥ 3 years

Diagnosis	Sex	AF < 3 years SR < 1 month	AF < 3 years SR ≥ 1 month	AF ≥ 3 years SR < 1 month	AF ≥ 3 years SR ≥ 1 month
RHD	Female	9	20	13	0 $p < 0.001$
	Male	4	10	3	0
Total		13	30	16	0 $p < 0.001$
Without RHD	Female	2	6	0	1
	Male	2	13	4	0 $p < 0.01$
Total		4	19	4	1 $p < 0.05$
Grand total		17	49	20	1 $p < 0.001$

The p values refer to χ^2 tests

Table 24 Patients distributed according to length of sinus rhythm RHD without RHD sex and duration of atrial fibrillation < 6 months and ≥ 6 months

Diagnosis	Sex	AF < 6 months SR < 1 month	AF < 6 months SR ≥ 1 month	AF ≥ 6 months SR < 1 month	AF ≥ 6 months SR ≥ 1 month
RHD	Female	2	8	18	6 $p < 0.01$
	Male	2	4	3	7
Total		4	12	21	13 $p < 0.05$
Without RHD	Female	0	3	3	2
	Male	0	7	7	6
Total		0	6	10	8
Grand total		4	18	31	21 $p < 0.01$

The p values refer to χ^2 tests

The group with atrial fibrillation for less than 3 years maintained sinus rhythm for considerably varying periods of time, on the average the sinus rhythm lasted for 491 days SE 93 range 1-2412 days

A comparison between the 13 converted women with RHD and atrial fibrillation for more than 3 years and the 28 converted women with RHD and a verified duration of atrial fibrillation of less than 3 years using Student's t-test showed no difference in age or relative cardiac size The series with more than 3 years' duration of atrial fibrillation

needed, however a significantly higher mean concentration of quinidine in the plasma for conversion than the others, 6 2 and 5 0 mg/l, respectively $p < 0.02$ The other patient series with more than 3 years duration of fibrillation were too small for statistical comparisons

It has been shown previously in this series that the percentage of successful conversions is significantly higher for patients who have had a duration of atrial fibrillation for less than 6 months

Patients with RHD and atrial fibrillation for less than 6 months maintain sinus rhythm better than those with duration of atrial fibrillation longer than 6 months Patients with sinus rhythm for less than 1 month were compared with those who had sinus rhythm for 1 month or more See table 24 $p < 0.05$

Patients without RHD and with a duration of atrial fibrillation of less than 3 years maintained sinus rhythm significantly better than patients with atrial fibrillation for 3 years or more, $p < 0.05$ Patients with sinus rhythm for less than one month were compared with patients who had had sinus rhythm for 1 month or more, see Table 23

On the average the 4 men with atrial fibrillation for 3 years or more maintained sinus rhythm for 14 days range 1—28 days Owing to a long interval between controls in 2 cases the possible maximum sinus rhythm could have been an average of 86 days range 8—221 days The woman with atrial fibrillation for more than 3 years had sinus rhythm for 286 days maximum 1 year and 7 days

In patients without RHD and with atrial fibrillation for less than 6 months and patients with atrial fibrillation for 6 months or more, a comparison was made between sinus rhythm for less than 1 month and sinus rhythm for 1 month or more No significant difference was found, see Table 24 It should be noticed in this connection that the patient group with fibrillation for less than 6 months is small 3 women and 3 men The reason for this is that it is difficult to establish the onset of fibrillation in patients without RHD

Roentgenological relative cardiac size

With respect to roentgenological, relative cardiac size there was no difference in distribution between the number of patients who maintained sinus rhythm for less than 1 month and those who maintained it for 1 month or more This applied both to patients with and without RHD as well as for the whole series Patients who had a relative cardiac size of less than 600 ml per m^2 BSA were compared with those who had 600 ml or more All patients with a duration of fibrillation of 3 years or more were excluded at the χ^2 test

Functional groups

There were no significant differences between patients in functional groups I+II and III+IV with regard to maintenance of sinus rhythm for less than 1 month or more than 1 month This applied to the total material as well as to patients with RHD or without RHD Table 25

Table 25 Patients distributed according to RHD, without RHD, sex, functional group and length of sinus rhythm

Diagnosis	Sex	Duration of SR	Functional group I	II	III	IV	Total
RHD	Female	< 1 month	2	12	11	2	
		≥ 1 month	2	7	12	3	
							51
RHD	Male	< 1 month	3	3	2	1	
		≥ 1 month	2	4	8	0	
							23
Without RHD	Female	< 1 month	2	5	3	1	
		≥ 1 month	1	4	6	4	
							26
Without RHD	Male	< 1 month	3	6	3	3	
		≥ 1 month	7	9	11	[illegible]	
							48
	Total	< 1 month	10	26	19	7	
		≥ 1 month	12	24	37	13	
Grand total			22	50	56	20	148

Maintenance dose of quinidine during the first month after conversion

Group I which received ≥ 1.2 g quinidine sulphate per day throughout the first month after the conversion continuously or until relapse to atrial fibrillation comprised 84 patients (8 with a duration of the fibrillation of ≥ 3 years)

Group II which received < 1.2 g quinidine sulphate per day during the first month comprised 38 patients (7 with a duration of the fibrillation of ≥ 3 years). The original dose had been reduced at various points in time during the first month mainly because of side effects or for some other reason

Group III in which the quinidine was discontinued at various points *in time during the first month* because of severe side effects comprised 20 patients (3 with a duration of the fibrillation of ≥ 3 years), see Table 26. In this group the quinidine had been replaced by procaine amide (250 mg 4—6 times daily) in 9 cases

Table 26 *Patients in Group III distributed according to main causes for discontinuing the maintenance dose of quinidine*

Side effects	Quinidine discontinued 1st month No. of pat.	Quinidine discontinued 2nd–28th month No. of pat.
Syncope	6	0
Fever	9	0
Vomiting	2	0
Diarrhea	1	0
Thrombocytopenia	2	2
	20	2

Table 27 *Patients distributed according to length of sinus rhythm, RHD, without RHD, sex and maintenance dose of quinidine*

Diagnosis	Sex	I Quinidine sulphate ≥ 1.2 g/day SR < 1 month	I SR ≥ 1 month	II Quinidine sulphate < 1.2 g/day SR < 1 month	II SR ≥ 1 month	III Quinidine sulphate 0 g/day SR < 1 month	III SR ≥ 1 month	
RHD	Female	3	14	4	9	6	1	
	Male	5	10	1	3	0	1	
Total		8	24	5	12	6	2	I–III $p < 0.05$
Without RHD	Female	7	8	2	4	2	2	
	Male	6	23	2	6	2	3	
Total		13	31	4	10	4	5	
Grand total		21	55	9	22	10	7	I–III $p < 0.05$

The p values refer to χ^2 tests.
Patients with atrial fibrillation duration ≥ 3 years have been excluded.

In 6 patients the quinidine was discontinued because of some other arrhythmia: in 5 because of flutter and in 1 because of nodal rhythm. These patients are not included in Group III. Four patients with flutter converted to atrial fibrillation after the discontinuation of the quinidine while one patient retained flutter. The patient with nodal rhythm converted to sinus rhythm.

A comparison between Groups I and III involving patients with RHD showed that there was a significantly better retention of sinus rhythm in Group I for patients with sinus rhythm for 1 month or more (see Table 27, $p < 0.05$). Patients with more than 3 years' fibrillation were not included in this calculation.

There was the same trend between Groups II and III but there was no signi

ficant difference with respect to maintained sinus rhythm, nor was there any significant difference between Groups I and II in this respect

When the same comparisons were made in patients without RHD no significant differences were found

For the total patient series a comparison showed that there was a significantly longer retention of sinus rhythm in Group I than in Group III A comparison between Group I and Groups II+III showed that there was no difference in this respect, see Table 27

In the 20 patients who discontinued their quinidine therapy during the first month after the conversion — all because of side effects — it was possible to establish that there was a definite relapse to atrial fibrillation in 12 patients within 36 days Of these 10 had RHD and 2 did *not*

Six patients retained sinus rhythm without quinidine for 5, 8 and 9 months and 4, 6 and 7 years One patient had RHD while the others did not Two patients retained sinus rhythm for an unknown time

The verified duration of atrial fibrillation in those six patients was in three cases 23 days, and in the others 1 month 3 months and 11 months As it has been impossible to establish the exact onset of fibrillation it cannot be stated with certainty that these patients had a short duration of fibrillation

The patients with 6 and 7 years' maintained sinus rhythm were cases of treated hyperthyroidism

Maintenance dose of quinidine during the 2nd and the 28th month after conversion

Group I Thirty-six patients received ≧ 1 2 g quinidine sulphate per day continuously or until there was a relapse to atrial fibrillation between the 2nd and the 28th month after the conversion (13 with and 23 without RHD) One more patient with this dose died during sinus rhythm and 2 were not observed during this period

Group II Twenty-eight patients received < 1 2 g quinidine sulphate per day for varying periods of time continuously or until there was a relapse to atrial fibrillation (19 of these had RHD 9 had not) One additional patient with this dose died and one patient was not observed

Group III Nine patients were started on quinidine, but stopped taking the medicine for one reason or another No patient had had any quinidine within 3 months of the time they were observed In 2 of these, quinidine was discontinued because of thrombocytopenia Three patients discontinued the quinidine therapy themselves although they had no side effects and in 4 the quinidine was discontinued by a physician for unknown reasons

Table 28 Patients distributed according to length of sinus rhythm, RHD, without RHD and maintenance dose of quinidine during the 2nd to 28th month after conversion

Diagnosis	I Quinidine sulphate > 1.2 g/day SR < 28 months	SR ≥ 28 months	II Quinidine sulphate < 1.2 g/day SR < 28 months	SR ≥ 28 months	III Quinidine sulphate 0 g/day SR < 28 months	SR ≥ 28 months	
RHD	6	7	13	6	4	0	
Without RHD	1	16	4	5	2	3	
Total	13	23	17	11	6	3	I–II+III $p < 0.05$

The p value refers to a χ^2 test

Table 29 Mean maximum concentrations of quinidine in plasma and coefficients of variation in 12 patients during maintenance therapy with quinidine Durules® Observations between 3 weeks to 25 months after start of therapy

Daily dose of quinidine Durules® g	Mean maximum concentration of quinidine in plasma mg/l	No of observations	Coefficient of variation per cent
0.8	2.0	5	21.4
1.2	2.2	6	15.?
1.2	2.7	5	19.1
1.2	2.8	9	14.8
1.2	3.0	5	9.7
1.2	4.0	5	21.6
1.6	1.8	6	6.6
1.6	2.3	5	24.8
1.6	2.4	4	7.4
1.6	4.2	7	17.4
2.0	3.6	7	10.9
2.0	5.1	5	8.1

One more patient had to discontinue the quinidine because of nodal extrasystoles but he was not observed for 28 months

When comparing Group I with Groups II+III there was a significantly longer maintenance of sinus rhythm in Group I ($p < 0.05$, Table 28). A comparison between Groups I and II showed that there was no significant difference in this respect

Comparisons between Groups I and III and Group I and Groups II+III showed that there was no significant difference in maintained sinus rhythm between patients with RHD and patients without RHD. A sinus rhythm duration

of < 28 months was compared with a duration of ≧ 28 months, see Table 28

Of the 9 patients who discontinued their quinidine therapy between the 2nd and the 28th month after the conversion, 2 relapsed to atrial fibrillation within 18 days and 6 patients maintained a verified sinus rhythm between 4 months and 3 years without quinidine One patient had RHD, 5 did not One patient was not observed after the discontinuation of the quinidine therapy

Quinidine concentration in plasma

In 12 patients the maximum concentration of quinidine in plasma during the day was determined 4 hours after the morning dose of quinidine Durules® It was controlled several times during the follow up period The concentration varied individually after the same dose but in any given patient it was remarkably constant with low coefficients of variation See Table 29

Complications and side effects

Gastro-intestinal side effects

Of the 148 converted patients, 61 had gastro-intestinal side effects on a maintenance dose of quinidine after the first conversion In 26 patients with nausea, there were 15 cases combined with diarrhea while 35 patients had diarrhea alone In spite of these disorders, 26 patients were able to continue with the same dose of quinidine In 22 patients, the quinidine dose was reduced and it was discontinued altogether in 13 patients

In this last group, the disorders were most often combined with other side effects fever in 5 cases, thrombocytopenia in 3 and syncope in 2

Thrombocytopenia

In 148 converted patients we found thrombocytopenic purpura in 3 cases and an increased bleeding tendency with hematoma in one additional case during the follow up period using quinidine as maintenance therapy The lowest platelet count observed was 6000/mm³ The patient with this low value also had bleeding into the gastro intestinal tract in addition to general purpura The other patients with purpura or hematoma had platelet counts which were never below 12 000, 15 000 and 25 000 platelets/mm³ In all cases, the platelet levels increased to normal values after discontinuation of the quinidine One patient had had quinidine combined with chlorthalidone which also was discontinued

The platelets were checked in 77 converted patients during the follow-up period Among these, we found platelet counts below 100 000/mm³ in a total of 26 patients, i e in 34% Eight patients had platelet counts of less than 30 000/mm³

In 10 patients, thrombocytopenia occurred within one month and in the others between 2 months and 2 years of the quinidine administration In 19 patients, we found thrombocytopenia after the first conversion but in an additional 7 patients it was not observed until after a repeated quinidine conversion

Of the 26 patients, 10 took thiazide preparations or chlorthalidone together with quinidine while one patient took quinidine and procaine amide In one of these cases, the platelet value was low even before the administration of quinidine, it was reduced further during the quinidine therapy

The administration of quinidine was discontinued in 15 patients of whom 3 had already reverted to atrial fibrillation In 4 cases procaine amide was substituted for quinidine At the same time the previously mentioned diuretics were discontinued in 7 cases

All 77 patients were not checked equally often and regularly with respect to the platelet counts during the whole follow up period It is therefore possible that there may have been additional cases of thrombocytopenia which did not show clinical signs of an increased bleeding tendency

Leukopenia and urticaria

On the 12th day after conversion to sinus rhythm, one patient on a maintenance dose of quinidine Durules® 0 8 g twice daily began to have urticaria with itching on his trunk, arms and legs as well as *fever (38° C) His white blood*-count was low, but never lower than 1080 per mm³ with a pronounced shift to the left in the differential count His platelet count was normal 200 000/mm³ The patient's condition improved rapidly when the quinidine was discontinued and treatment with antazoline and cortisone was started This patient reverted to atrial fibrillation

ECG changes

P-R interval > 0 22 sec on one, and most often several controls after the first conversion was recorded in 36 of 148 converted patients, 25 with and 11 without RHD Such an interval occurred significantly more often in patients with RHD, $p < 0.02$ As a rule, the quinidine dose was not reduced for this reason In occasional cases the digitalis dose was reduced

Arrhythmias other than chronic atrial fibrillation (defined as atrial fibrillation for more than one week) verified by means of ECG occurred in 30 of 148 patients after the first conversion The distribution of the ECG changes is shown in Table 30 In addition, 17 patients reported subjective signs of tachycardia or irregular rhythm (extra systoles?) for a period shorter than 7 days

QRS > 0 10 sec was observed in all 5 patients with converted atrial fibrillation and bundle branch block In these, the bundle branch block remained during sinus rhythm One of the patients alternated between bundle branch block and a normal QRS interval from time to time both during atrial fibrillation and sinus rhythm After conversion there was one additional patient without RHD whose *QRS complex increased to 0 12* sec This remained until he relapsed to atrial fibrillation with a normal QRS without quinidine

Arterial emboli in patients with RHD

Of the 132 patients with RHD, 9 had sinus rhythm throughout the observation period and one patient had atrial flutter The other 122 patients had atrial fibril

Table 30. Converted patients with arrhythmias less than 7 days during the follow up period

Diagnosis	Sex	ES	Nodal rhythm	Atrial flutter	Escaped beats	Sinus arrhythmia	Idio-V rhythm	Total
RHD	Female	0	4	1	2	0	1	8
	Male	4	0	1	0	1	0	[illegible]
Without RHD	Female	3	1	0	1	0	0	5
	Male	6	1	3	1	0	[illegible]	11
Total		13	6	5	4	1	1	30

lation during some part of the follow up period in spite of new conversion attempts by means of quinidine or DC shock.

In 26 of these 122 patients, 18 women and 8 men, there was a total of 37 peripheral arterial emboli. In all cases of embolus the patient had atrial fibrillation. Not one of the emboli occurred in direct connection with relapse to atrial fibrillation with the possible exception of one patient in whom the time interval, however, was impossible to determine.

The distribution of emboli was 21 cerebral emboli, 8 to the iliac artery or its branches, 4 to the mesenteric artery, 3 to the renal artery and one to the brachial artery.

Eleven of the 26 patients, 10 women and 1 man, had had one or more peripheral emboli during atrial fibrillation also before the conversion attempt.

All patients in whom emboli occurred had mitral disease. Twenty of them had non-operated mitral stenosis, 13 of which were combined with mitral insufficiency or aortic disease. Five patients had operated mitral stenosis. In one patient, there was mitral insufficiency combined with aortic disease.

Of the 122 patients with RHD and atrial fibrillation, 68 patients, 47 women and 21 men, received dicoumarol for varying periods of time in order to prevent the occurrence of emboli. In 14 cases, the dicoumarol was administered because of a peripheral embolus. In the other cases, the dicoumarol therapy was continued immediately after unsuccessful conversion attempts or it was given when there was a relapse to atrial fibrillation.

During dicoumarol therapy there were 11 embolic episodes. The prothrombin index is known in 7 of these cases and in all of them it was high (65, 71, 73, 75, 78, 87 and 95 units according to the method of Lehmann, 1942).

Thus, there were 4 possible emboli at a prothrombin index below 40 units. Of the other 33 emboli, 26 occurred in patients who had not been treated with dicoumarol while 7 occurred in patients in whom there had been insufficient dicoumarol therapy with a high prothrombin index. Table 31.

Table 31 Distribution of patients with RHD according to frequency of the first arterial embolus and treatment with dicoumarol

Treatment		Emboli	No emboli	Total	
Dicoumarol		3	52	55	$p < 0.001$
No dicoumarol	16 }	23	44	67	
Insufficient dicoumarol	7 }				
Total		26	96	122	

The p value refers to a χ^2 test.

Arterial emboli in patients without RHD

Of 105 patients without RHD 24 had sinus rhythm throughout the observation period while 81 had atrial fibrillation during the follow up period. In 8 patients there was a total of 8 peripheral emboli, all of which were cerebral and all of which occurred during atrial fibrillation.

Three of these 8 patients had had a previous cerebral embolus prior to the conversion attempt. All 3 had been converted to sinus rhythm but had relapsed to atrial fibrillation. One of the patients had his second embolus in direct connection with a relapse to atrial fibrillation after 5 years and 2 months of sinus rhythm. The other two patients had their second embolus after a period of atrial fibrillation of 2 years and 1 month each.

There were 5 additional cerebral emboli in patients who had not had any embolus prior to the conversion attempt. One of these had had sinus rhythm for one day and one sinus rhythm for one month. The first patient had relapsed to atrial fibrillation 11 months, the other 3 weeks prior to the embolus. It was not possible to convert the other three patients to sinus rhythm.

After an unsuccessful conversion attempt or a relapse to atrial fibrillation, 16 of the 81 patients without RHD were given dicoumarol as prophylaxis against the occurrence of embolus for various periods of time. There was no peripheral embolus in any of these patients during atrial fibrillation although 4 of them had had cerebral embolus prior to the administration of dicoumarol. None of the 8 patients who had cerebral emboli had been given dicoumarol therapy.

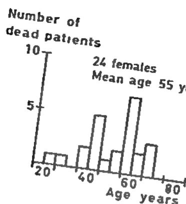
Mortality

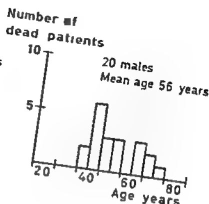
Of the 132 patients with RHD 44, i.e. 33% died during the observation period. The mean age of these patients, of whom 24 were women and 20 were men, was 56 years. The age distribution is shown in Fig. 5.

Immediately prior to death, 2 women had sinus rhythm and were on a maintenance therapy of quinidine. Both women had operated mitral stenosis. One had recently been subjected to DC countershock and the other had attacks of atrial flutter. They died in their sleep. The other 42 patients had atrial fibrillation prior to the time of death and were not being treated with quinidine.

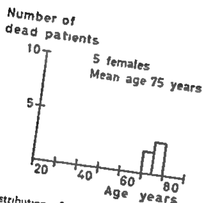
The immediate cause of death is shown in Table 32.

RHD





Without RHD



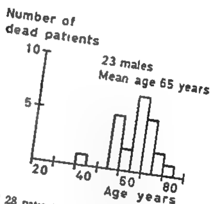


Fig 5 Age distribution of 44 patients with RHD and 28 patients without RHD who died during the observation period

In 3 cases an autopsy showed that the left ventricle was largely occluded by thrombus formation Two of these patients died with symptoms characteristic of cardiac decompensation while one suffered sudden death

Of 105 patients without RHD 28 i e 27% died during the observation period This group comprised 5 women and 23 men The mean age of the deceased was 67 years The age distribution is shown in Fig 5 Of these patients, 18 had been converted to sinus rhythm, but 16 of them had relapsed to atrial fibrillation

Immediately prior to death, 2 patients had sinus rhythm and were taking quinidine One had attacks of atrial flutter Both died suddenly The other 26 patients had atrial fibrillation and were not taking quinidine at the time of death

Table 32 Number of deceased patients cau e of death and number of patients on whom autopsy was performed

	No of pat		No of pat autopsied	
	RHD	Without RHD	RHD	Without RHD
Peripheral arterial emboli	8 (5 cerebral) (3 mesenteric)	(2 cerebral)	[illegible]	2
Sudden death	14	13	9	2
Heart failure	12	3	12	3
Myocardial infarction	—	4	—	4
Pulmonary embolism	1	—	1	—
Septicemia	1	—	1	—
Retroperitoneal bleeding	1	—	1	—
Bronchopneumonia	4	1	4	1
Nephrosclerosis	—	1	—	1
Malignant lymphogranulomatosis	—	1	—	1
Cancer of the stomach	2 (1 cerebral embolus)	—	1	—
Cancer of the prostate	—	1	—	1
Cancer of the lung	—	1	—	1
Penetrating knife wound of the thorax	—	1	—	1
Reason unknown	1	—	1	—
Total	44	28	36	17
Grand total	72		[illegible]	

Comments

Analysis of maintained sinus rhythm

Most relapses to atrial fibrillation occurred during the first month which has previously been pointed out Cramér 1963, Rokseth 1963

As for the follow-up investigation, there is no comparable unselected large quinidine converted patient series except for that of Rokseth 1963 He reports that most of his early relapses to atrial fibrillation 23 of 29 occurred before the patient had been released from the hospital In addition to this, he gives results 6 months after the conversion Thus our results are not directly comparable when analyzing the factors which affect an early relapse to sinus rhythm Rokseth used a maintenance dose of quinidine of the same magnitude as the one used by us 0 3 g quinidine sulphate 3 or 4 times daily

Rokseth reported that 65 of 107 converted patients i e 69%, had sinus rhythm after 6 months and that 13 patients had not been observed Our corresponding figures are 48% and 1 patient who was not observed

In Radford and Evans DC converted patients 2 years after conversion 13 per

cent still had sinus rhythm. Compared to 33 per cent in our series. This can be due to a selection of quinidine-tolerant patients in our series and/or that Radford and Evans did not use prophylactic quinidine more than 5 months after the conversion.

Sex, age and cardiac size

There was no relation between sex, age, and roentgenological relative cardiac size and maintained sinus rhythm during the first month in our series. This is in good agreement with Rokseth's findings as regards age. Rokseth does not discuss the other factors.

Diagnosis

In Rokseth's material, there was no difference in maintained sinus rhythm between patients with and patients without RHD. Our data on the first month after the conversion are in agreement with this.

Duration of atrial fibrillation

Duration of atrial fibrillation for more than 2 years compared with less than 2 years showed a pronounced difference in maintained sinus rhythm in Rokseth's series. This too agrees with our analysis of the first month after the conversion during which 20 of 21 patients with atrial fibrillation for more than 3 years relapsed to atrial fibrillation within one month. The importance of the duration of fibrillation with respect to maintained sinus rhythm was pointed out as early as 1923 by Viko *et al.*

Our series also shows that patients with RHD and atrial fibrillation for less than 6 months retain sinus rhythm significantly better than when the atrial fibrillation has lasted for more than 6 months.

The reason for the negative influence of a long lasting fibrillation both on the conversion frequency and the ability to maintain sinus rhythm has not been determined. It should be noted, however, that patients with more than 3 years duration of atrial fibrillation required higher quinidine levels in plasma for conversion than the others.

Functional groups

In Rokseth's series, functional group IV had a greater tendency towards relapse to atrial fibrillation than functional group I–III. This does not agree with our material in which functional groups I+II did not differ from functional groups III+IV with respect to maintained sinus rhythm during the first month.

Quinidine as maintenance dose

In our material, there was a significantly better retention of sinus rhythm in patients with RHD and totally with a quinidine sulphate dose of ≧ 1.2 g/day than in the group in which the quinidine was discontinued during the first month because of side effects.

The patients with quinidine sulphate ≧ 1.2 g/day had a significantly higher frequency of maintained sinus rhythm for 2–28 months after the conversion than the patients who had a lower quinidine dose or no quinidine at all. Both of these facts indicate that quinidine is of importance with respect to maintaining

sinus rhythm not only for the immediate post conversion period but also for long-terms results

There is little information in the literature as to relapses to atrial fibrillation after discontinuation of quinidine Sokolow and Ball, 1956 reported that in their selected material 85% of 42 patients relapsed to atrial fibrillation without quinidine, most of them within one week

This does not agree with our findings in 20 patients in whom the quinidine was discontinued during the first month after the conversion Six of these maintained sinus rhythm more than 5 months after this

Engstrom, 1967, recently showed that 2 groups of patients without RHD who were observed for 3 months maintained sinus rhythm equally well regardless of whether they had taken quinidine or not The importance of quinidine with respect to maintenance of sinus rhythm in such patients can thus be discussed

Arterial emboli

The risk of embolus during atrial fibrillation is small except for atrial fibrillation combined with RHD especially mitral disease in which cases the risk is great, Askey, 1962 The distribution of the peripheral emboli in the present series supports this finding Thirty seven emboli occurred in 26 patients with mitral disease while there were 8 emboli in 8 patients without RHD

During a follow up period of at least 28 months in 233 patients all 45 emboli occurred during atrial fibrillation while there was no embolus during sinus rhythm This indicates strongly that sinus rhythm helps to prevent embolus contrary to the assumption made by Askey, 1962

Sixty-eight of our patients were treated periodically with dicoumarol There was a lower frequency of embolus in those patients with RHD who can be assumed to have been given an adequate treatment with dicoumarol These patients were compared with other patients with RHD without dicoumarol or with a clearly insufficient dose of dicoumarol This seems to support the fact that dicoumarol treatment has a favorable effect with respect to preventing embolus in patients with atrial fibrillation and RHD which agrees with the assumption made by Askey, 1962

Mortality

During a follow-up period of at least 28 months 4 of 72 deceased patients in a series of 233 patients died a sudden death during sinus rhythm while on maintenance therapy with quinidine The other 69 patients died during atrial fibrillation whithout quinidine Of these 69 deaths 23 were sudden deaths and an additional 10 were caused by peripheral arterial embolus

Since in this material of patients suffering from severe heart disease as many as 23 died suddenly without quinidine it is difficult to ascribe the 4 sudden deaths in patients who had received quinidine to this medication Only one of these 4 patients was autopsied This post mortem examination revealed an advanced re stenosis of the mitral valve

Gastro-intestinal side effects

Of the 61 patients who had nausea or diarrhea as a result of the quinidine it was necessary in many cases to reduce the quinidine dose Moderate diarrhea alone, often changed spontaneously to normal bowel habits after quinidine administration for some time

It is difficult to determine whether the gastro-intestinal side effects were directly caused by the magnitude of the quinidine dose as the patients' subjective difficulties varied from time to time though the quinidine dose was unchanged

Thrombocytopenia

Thrombocytopenia during quinidine treatment has been described several times in the literature, e g by Bolton and Dameshek, 1956, and Stratford and Tanaka 1965 Goodman and Gilman, 1965, report that thrombocytopenic purpura is a rare complication In our series there were only 3 cases of purpura

In the 26 cases of thrombocytopenia which were found in our 77 controlled patients there were 10 spontaneous remissions after continued quinidine therapy including, in 2 cases thiazide preparations In 5 other cases, the quinidine and the thiazide preparation were discontinued simultaneously which makes it impossible to determine the role played by quinidine alone in the genesis of thrombocytopenia In 10 patients only the quinidine was discontinued and a remission of thrombocytopenia was obtained In these, quinidine may have caused the thrombocytopenia but no provocative tests were performed

Leukopenia and urticaria

The combination of leukopenia urticaria and fever which was found in one case appears to be one of the more rare side effects found during quinidine therapy Cases of urticaria alone have been described previously by Siegal and Horn, 1950

ECG changes

Prolonged atrio ventricular conduction time during quinidine treatment was reported by Frey, 1918 and Ellis 1921 In our series the frequency is relatively high Of the converted patients 24% had a lengthening of the P R interval to > 0 22 seconds during the follow-up period This occurred significantly more often in patients with RHD who could have had rheumatic, inflammatory injury in the conduction system Rokseth 1963 does not give any information as to the conduction time in the patients he followed up

Comparisons between different states in atrial fibrillation and in sinus rhythm in the same patients

To get an objective measure of the possible favorable effects of conversion the following examinations were performed in the same patients both during atrial fibrillation and during sinus rhythm heart X-ray, ECG during exercise and hemodynamic determinations at rest and during exercise

Roentgenological relative cardiacsize during sinus rhythm and atrial fibrillation

An investigation of the roentgenological relative cardiac size in 13 well-digitalized patients in the prone position showed that there was a significant difference with a larger cardiac size during sinus rhythm than during atrial fibrillation $p < 0.05$ Two of these patients were on diuretic therapy The weights of the patients showed no significant difference between the first and the second X-ray examination. The mean value during atrial fibrillation was 650 ml/m² BSA, and during sinus rhythm 667 ml/m² BSA, SE for the difference was 7 ml

Six patients were examined during atrial fibrillation and 6—12 months after conversion Two patients had RHD and 4 had no RHD No significant difference in relative cardiac size in the prone position was found between the two X-ray examinations

ECG during exercise during sinus rhythm and atrial fibrillation

An ECG on patients exercising on a bicycle ergometer was taken by the Clinical Physiological Laboratory both while the patient had atrial fibrillation and after conversion to sinus rhythm Twenty six patients were investigated in this manner

In 11 women with RHD and atrial fibrillation an exercise ECG was taken before and 1—18 days after the conversion to sinus rhythm In 4 the digitalis dose was reduced during sinus rhythm One patient was on a mercurial diuretic prior to the first examination and was on chlorothiazide therapy at the time of the second examination

In one patient only ECG at rest before and after conversion was taken because of ECG changes in the form of a pathological Q wave

Calculated on the basis of the individual difference between the value during atrial fibrillation and the value during sinus rhythm the ECG did not show any difference in ventricular rate at rest

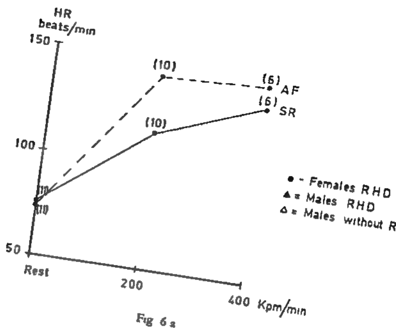


Fig 6 a

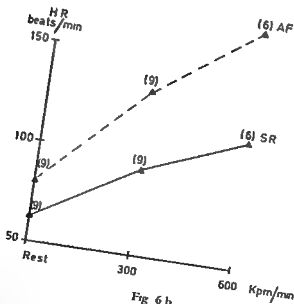


Fig 6 b

Fig 6 a b c Heart rate as measured on the ECG at rest and during exercise Comparison of mean values in the same patients during atrial fibrillation and during sinus rhythm
Figures in parentheses denote the number of patients

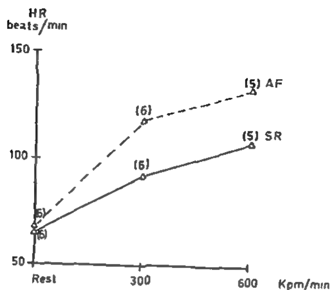


Fig 6 c

Table 33 Heart rates during exercise in atrial fibrillation and in sinus rhythm in the same patients

Diagnosis	Sex	Rest No of pat	Rest Heart rate mean beats/min AF	Rest Heart rate mean beats/min SR	Rest SE of diff	Exercise 200 kpm/min Female, 300 kpm/min Male No of pat	Heart rate mean beats/min AF	Heart rate mean beats/min SR	SE of diff		Exercise 400 kpm/min Female, 600 kpm/min Male No of pat	Heart rate mean beats/min AF	Heart rate mean beats/min SR	SE of diff	
RHD	Female	11	74	75	5	10	141	115	5	$p < 0.001$	6	143	134	7	
	Male	9	80	62	9	9	131	92	11	$p < 0.01$	6	167	112	7	p < 0 0
Without RHD	Male	6	68	65	6	6	118	92	11		5	132	108	9	
Total		26	75	68	4	25	132	101	5	$p < 0.001$	17	148	119	6	p < 0 0

■ values refer to t tests of the mean difference (diff) of the paired differences

When the patient was exercising at 200 kpm/min there was a significantly lower ventricular rate during sinus rhythm than during atrial fibrillation $p < 0.001$ see Fig 6 and Table 33 When the exercise was 400 kpm/min there was no significant difference between ventricular rate during sinus

rhythm and ventricular rate during atrial fibrillation Only 6 patients were able to perform this work load

In 9 men with RHD and atrial fibrillation, an exercise ECG was taken before and 1—21 days after the conversion to sinus rhythm Only one patient was on diuretic therapy He was receiving the same dose of chlorothiazide at the time of the two examinations In 4 of the the cases the digitalis dose was reduced while in one case it was increased during sinus rhythm

Calculated on the basis of the individual difference between the value during atrial fibrillation and the value during sinus rhythm, there was no difference in ventricular rate at rest At exercise, there was a significantly lower ventricular rate during sinus rhythm than during atrial fibrillation both at 300 and 600 kpm/min see Fig 6 and Table 33, $p < 0\ 01$ and $< 0\ 001$ respectively

In 6 men without RHD an exercise ECG was taken during atrial firillation before and 2—4 days after the conversion to sinus rhythm No patient received any diuretic The digitalis dose was not changed between the times of the examination except in one patient in whom it was discontinued for 2 days during sinus rhythm because of diarrhea In this patient the dose was changed from acetyldigitoxin 0 2 mg daily to lanatoside C 0 25 mg twice daily

Calculated on the basis of the individual difference between the value during atrial fibrillation and the value during sinus rhythm an ECG at rest or at exercise 300 and 600 kpm/min showed no significant difference in ventricular rate but there was a tendency towards a lower ventricular rate at exercise during sinus rhythm see Fig 6 and Table 33

For the whole series, 26 patients, there was no difference in ventricular rate at rest during atrial fibrillation as compared with ventricular rate during sinus rhythm

At slight exercise, 200 kpm/min for the women and 300 kpm/min for the men, there was a significantly lower ventricular rate during sinus rhythm $p < 0\ 001$

At heavier exercise, 400 kpm/min for the women and 600 kpm/min for the men, there also was a significantly lower ventricular rate during sinus rhythm than during atrial fibrillation $p < 0\ 001$

Hemodynamics during sinus rhythm and atrial fibrillation

Cardiac output determination, ECG registration and intraarterial pressure determination with the patient at rest in a chair were performed both during atrial fibrillation and sinus rhythm on the average 10 days after conversion in 23 digitalized patients In addition in one patient both examinations were made not only before but also after operation for mitral stenosis The oxygen consumption was determined simultaneously in 16 of these patients

Of these patients 14 (5 women and 9 men) had RHD and 9 men had atrial fibrillation without RHD

At the examination during sinus rhythm the digitalis dose was unchanged in 12 cases reduced in 9 cases and in

Tab 34 H modynam c aata dur ng atr al f brilla on and nus rhythm in the ame pat nts

	Diagnosis	Sex	Rest: No of pat	Rest: Mean AF	Rest: Mean SR	Rest: SE of diff	Exercise 200 kpm min: No of pat	Exercise: Mean AF	Exercise: Mean SR	Exercise: SE of diff
CO l min	RHD	Female	(5)	3.2	3 8	0.2 p < 0 05	(3)	5.5	6 8	0 8
		Male	(10)	4 0	4 7	0 2 p < 0 01	(5)	6.5	7 7	0 7
	W thout RHD	Male	(9)	4 7	6 4	0.2 p < 0 001	(7)	7 0	8 9	0 4 p < 0 005
	Mean		(24)	4 1	5 ?	0.2 p < 0 001	(15)	6 6	8 1	0.3 p < 0 001
HR beats min	RHD	Female	(5)	73	65	4	(3)	133	122	3
		Male	(10)	73	6?	5	(5)	151	94	13 p < 0 0
	W thout RHD	Male	(9)	73	66	7	(7)	122	106	1?
	Mean		(24)	73	64	3 p < 0 0?	(15)	133	105	8 p < 0 005
SV ml	RHD	Female	(5)	44	58	2 p < 0 005	(3)	43	59	7
		Male	(10)	59	8	5 p < 0 01	(5)	45	81	7 p < 0 01
	W thout RHD	Male	(9)	69	98	10 p < 0 025	(?)	6?	86	11
	Mean		(24)	60	81	5 p < 0 001	(15)	53	9	6 p < 0 001
O_2 cons. ml/min	RHD	Female	(4)	261	242	11	( )			
		Male	(7)	258	241	11	(3)	96	865	80
	W thout RHD	Male	(6)	291	285	3	(4)	918	948	29
	Mean		(17)	2 0	257	5 p < 0 0.5	(7)	866	912	35
BA_S mm Hg	RHD	Female	(4)	165	144	7 p < 0 05	(2)	203	1 6	47
		Male	(8)	121	126	4	(3)	128	131	1?
	W thout RHD	Male	(?)	133	133	7	(6)	1 0	137	10 p < 0 05
	Mean		(19)	135	132	4	(11)	164	143	10
BA_D mm Hg	RHD	Female	(4)	6	64	4	(2)	105	89	6
		Male	(8)	73	69	2	(3)	—	68	9
	W thout RHD	Male	(7)	84	75	5	(6)	99	5	6 p < 0 0
	Mean		(19)	77	0	2 p < 0 005	(11)	94	6	4 p < 0 005
BA_M mm Hg	RHD	Female	(4)	102	9	6	(2	140	117	2
		Male	(8)	89	88	3	(3)	10	69	1 p < 0 0
	W thout RHD	Male	(7)	100	9	5	(6)	1.2	95	7 p < 0 0
	Mean		(19)	96	90	3	(11)	1?0	9	5 p < 0 00
Per pheral ascular es stance uni s	RHD	Female	(4)	32 8	25 1	1 3 p < 0 01	)	35	18 1	4 6
		Male	(8)	22 1	18 6	1 0 p < 0 02	(3)	15 0	11 8	4 1
	W thout RHD	Male	(7)	21 4	14 8	1 3 p < 0 005	(6)	1 6	11 0	1 p < 0 0
	Mean		(19)	4 1	18 6	0 8 p < 0 001	(11)	18 0	12.5	1.3 p < 0 005

P values refer t tes s of the mean difference (diff) of red differences

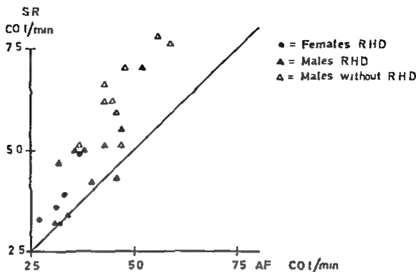


Fig 7 Cardiac output at rest during sinus rhythm in relation to cardiac output at rest during atrial fibrillation in the same patient The solid line is the line of identity

creased in 2 cases as compared with the doses given at the examination during atrial fibrillation One patient had been digitalized during atrial fibrillation with 1 2 mg acetyldigitoxin intravenously and the determinations had been made one hour later His maintenance dose during sinus rhythm was 0 2 mg acetyldigitoxin daily The same dosage of diuretic medicine of the thiazide type was being given to 4 patients at the two examinations The quinidine maintenance dose was 2 5 g daily in 2 cases, 1 6 g daily in 9 cases, 1 2 g in 6 cases, 0 9 g daily in one case while two patients received quinidine Durules®, 1 6 g daily and 2 4 g daily respectively

In three cases, the quinidine had been replaced by procaine amide 0 25 g 4 times daily because of vomiting or diarrhea.

Hemodynamics at rest

All the calculations were made on the basis of paired differences See Table 34 for values in patients with RHD, without RHD and values for the whole patient series Totally, the cardiac output at rest was significantly increased during sinus rhythm, $p < 0.001$ During atrial fibrillation the mean value was 4 1 liters per minute and during sinus rhythm 5 2 liters per minute

In the RHD group there were two patients who had an unchanged cardiac output and one whose cardiac output was reduced by 0 3 liter during sinus rhythm, see Fig 7

The ventricular rate at rest was reduced significantly during sinus rhythm, $p < 0.02$, although the digitalis dose was reduced in 9 cases In 7 patients there

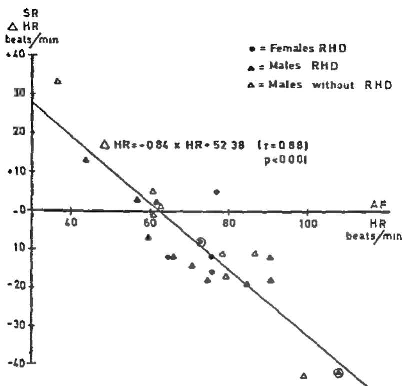


Fig 8 Change in ventricular rate (Δ HR) after conversion in relation to ventricular rate during atrial fibrillation at rest
Encircled symbols denote patients with increased digitalis dose after conversion

was an increased ventricular rate during sinus rhythm Six of these patients had an unchanged digitalis dose and one had a reduced digitalis dose during sinus rhythm All of the 3 patients with a ventricular rate below 60 during atrial fibrillation increased the rate during sinus rhythm The relationship between ventricular rate during atrial fibrillation and the change in ventricular rate by means of conversion to sinus rhythm in the individual patient is shown in Fig 8 $r = 0.88$ $p < 0.001$

For the whole series the stroke volume at rest increased significantly during sinus rhythm, $p < 0.001$ The mean value during atrial fibrillation was 60 ml and during sinus rhythm 91 ml

The oxygen consumption was reduced significantly during sinus rhythm, $p < 0.025$ The mean value during atrial fibrillation was 270 ml and during sinus rhythm 257 ml/min

For the whole series there was no significant difference between sinus rhythm and atrial fibrillation with respect to systolic pressure and the mean pressure in the brachial artery

The diastolic pressure was significantly lower during sinus rhythm, $p < 0.005$

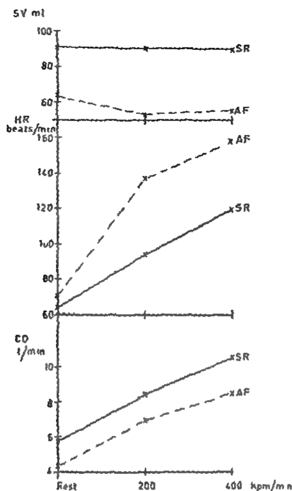


Fig 9 Stroke volume heart rate and cardiac output at rest and during exercise Mean values of the same men during atrial fibrillation and sinus rhythm (3 males with RHD and 4 without RHD)

Peripheral vascular resistance was significantly lower during sinus rhythm, $p < 0.001$ During atrial fibrillation the mean was 24 units and during sinus rhythm 19 units

Hemodynamics at exercise, 200 kpm per minute

All calculations were made on the basis of paired differences for the whole series see Table 34

The cardiac output was significantly higher during sinus rhythm $p < 0.001$, while the heart rate was significantly lower, $p < 0.005$ and the stroke volume was significantly higher, $p < 0.001$

There was no significant difference between sinus rhythm and atrial fibrillation with respect to systolic pressure The diastolic pressure the mean pressure and the peripheral vascular resistance

were significantly lower during sinus rhythm $p < 0.005$

The oxygen consumption, on the other hand tended to be higher during sinus rhythm but the difference was not statistically significant

Figure 9 shows the cardiac output, the ventricular rate and the stroke volume in 7 men 4 without RHD d i RHD who exercised both a c 400 kpm/min During atri tion there was no linear incr e tricular rate, which can be r tistically During sinus rhyth tricular rate increased to a and linearly

Comments

Roentgenological relative cardiac size

The roentgenological relative cardiac size of the heart, determined while the patient was in the prone position, was statistically significantly larger during sinus rhythm than during atrial fibrillation The difference, 17 ml, is hardly of any biological or clinical importance however After 6—12 months of sinus rhythm there was no significant difference compared with atrial fibrillation This may be due to the small number of patients in this series

Thus, the conversion did not result in a change in the form of reduced cardiac size neither immediately after the conversion nor on a long range basis

Heart rate during exercise

In 26 patients at rest there was no difference in heart rate when a comparison was made between sinus rhythm and atrial fibrillation in the same patients During exercise, the heart rate was significantly lower during sinus rhythm This observation has been made previously by Varnauskas *et al* 1959, on different patients in atrial fibrillation and sinus rhythm respectively

Hemodynamics

In the same 24 cases examined both during atrial fibrillation and after conversion to sinus rhythm, there was a significantly higher cardiac output during sinus rhythm while the patients were reclining and at rest although occasional patients had an unchanged or reduced cardiac output The heart rate was significantly lower during sinus rhythm while the stroke volume was significantly higher This indicates that there is a better hemodynamic adaptation after conversion to sinus rhythm At exercise on a bicycle ergometer these patients showed a higher cardiac output, a lower heart rate and a higher stroke volume during sinus rhythm than during atrial fibrillation The increase in the stroke work that this increase in the blood flow leads to is probably more than sufficiently compensated for by the improved peripheral blood flow and improved hemodynamic adaptation caused by the sinus rhythm especially during exercise As regards the values at rest, they are in good agreement with the results in 26 quinidine converted patients described by Rodman *et al*, 1966 One difference

was observed, however. In their series, it was the patients with arteriosclerotic heart disease and an almost normal cardiac output who had the smallest increase in the cardiac output. In our series on the other hand, 3 patients with RHD and a low cardiac output had an unchanged or reduced cardiac output during sinus rhythm. This is in agreement with Carleton and Graettinger, 1967. It may be referred to myocardial disease and ineffective atrial performance and in some patients the stenotic mitral valve may be the major factor.

According to Schroder, 1966, who used the same method as we did, quinidine in itself, in the same dosage as was used in the present series did not lead to any significant difference in the hemodynamics in patients reclining and at rest. McIntosh and Morris 1966 did not find that a plasma quinidine concentration of 3—4 mg/l affected either cardiac output or heart rate. Ferrer *et al* who in 1948 observed a reduction in the systolic pressure after quinidine in patients at rest used a one time dose of quinidine. Their findings are thus not representative for the probable hemodynamic adaptation to quinidine during a continuous maintenance therapy.

Thus there is no reason to assume that the observed changes during sinus rhythm in our series were caused by quinidine — on the contrary, they were caused by the normal rhythm.

Our series showed a linear, significant relationship between heart rate at rest during atrial fibrillation and the change of the frequency after conversion to sinus rhythm. Patients with a heart rate below 60 increased their rate while patients with a high rate reduced it. This must be an expression of the return to the frequency of the sinus node in all cases.

There was no linear increase in the heart rate during exercise while the patient had atrial fibrillation. A significant deviation from linearity could be observed. During the same exercise during sinus rhythm, the increase in the rate was smaller and linear.

Resnekov, 1967, recently described a linear relation between heart rate and work load in both atrial fibrillation and sinus rhythm during the same increasing work loads as we used on a bicycle ergometer. This may be due to different composition of the patient series. If however, the heart rate at rest in his series is taken into consideration in the calculations, there is a tendency towards the same pattern of additional increase in heart rate at the first work load in atrial fibrillation, as compared to sinus rhythm, as we have found. Resnekov also found increased cardiac output both at rest and during exercise in sinus rhythm, just as we did.

All conditions during exercise indicate that there is a better hemodynamic adaptation during sinus rhythm than during atrial fibrillation.

General discussion

Ever since therapy with quinidine was introduced, it has been argued as to whether the possible advantages of conversion to sinus rhythm in cases of atrial fibrillation compensate for the disadvantages and the risks. Opinions have varied and have often been based on patient series observed for a short period of time or on clinical findings alone. In spite of experience based on large, unselected patient series in recent years the indications for conversion of atrial fibrillation are still vague according to Rokseth 1963 and Sandoe *et al.*, 1965. Certain guide lines for selection of patients for conversion have been given by many authors and most recently by Lown, 1967, and Radford and Evans 1968.

The present investigation comprises a large, primarily unselected patient series and complements previous experience by a long and uniform observation period of 4 years with respect to maintained sinus rhythm. Furthermore the investigation gives information on the risk of embolus and mortality during a period of at least 28 months after the conversion attempt. The investigation also includes the hemodynamic consequences of conversion to sinus rhythm examined in the same patients during atrial fibrillation and sinus rhythm both at rest and during exercise in a sitting position on a bicycle ergometer under standard conditions. The examination during sinus rhythm was made after an average of 10 days after the conversion when the patient was on quinidine therapy.

The results of our investigation can be applied both to quinidine and DC countershock-converted patients. However, the conclusion concerning the maintenance of sinus rhythm after the conversion can be biased in favour of those converted with quinidine. Consider two series of patients, one converted with quinidine and the other with DC countershock, and both maintained on quinidine after the conversion. In the group converted with quinidine one finds only a few patients who do not tolerate quinidine, since these generally do not appear in the group converted. Therefore, those on a maintenance dose of quinidine after the conversion consist mainly of patients who can tolerate therapeutic doses of quinidine. This may not be the case for the DC countershock converted group, since those converted can be quinidine intolerant just as well as quinidine tolerant.

Subjective improvement

It is difficult to draw any definite conclusions as to a possible subjective improvement after the conversion on the basis of the descriptions by the patients The irregular rhythm with a varying stroke volume is experienced subjectively differently by the patients Some patients do not notice any difference between atrial fibrillation and sinus rhythm. The extent to which the heart rate can be controlled with digitalis during the fibrillation is of importance To say in advance which patients will be subjectively improved by conversion is difficult or impossible

Rokseth, 1963, reported a regularly occurring improvement after conversion, especially in patients with cardiac decompensation Maurice *et al*, 1956, also reported an almost consistently occurring improvement both in patients with cardiac decompensation and patients with functional heart disorders after conversion to sinus rhythm Goldman, 1951, Sokolow, 1951, and Hurst *et al* 1964, also described cases of lessened cardiac decompensation after conversion Most authors, however do not give any information on subjective improvement after conversion to sinus rhythm in the converted patient series

In our series some patients with rheumatic heart disorders, especially aortic disorders were clearly clinically improved during sinus rhythm than during atrial fibrillation One way in which this manifested itself was by the occurrence of cardiac decompensation when the sinus rhythm reverted to atrial fibrillation in spite of adequate medication with digitalis and diuretics Similarly, it manifested itself by a complete disappearance of the decompensation when the patient converted to sinus rhythm. In our series there were also patients who, after the conversion, no longer felt the heart beats and who for instance thus could sleep on their left side during sinus rhythm.

The determination as to whether there is a possible improvement during sinus rhythm is made difficult by the fact that many patients have side effects from the often complicated medication after having been converted to sinus rhythm This is particularly true for patients who are subjectively discomforted by the quinidine dose that has been considered necessary Sometimes, one is forced to use a therapy involving several preparations in the severely ill patients An optimal digitalis dose may be particularly difficult to obtain

In addition to this, the heart disease most often grows more severe during the observation time in patients with chronic heart disease It is therefore necessary to use large and comparable control groups to be able to determine whether a possible clinical improvement is obtained by means of conversion to sinus rhythm

C improvement

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was observed, however. In their series, it was the patients with arteriosclerotic heart disease and an almost normal cardiac output who had the smallest increase in the cardiac output. In our series, on the other hand, 3 patients with RHD and a low cardiac output had an unchanged or reduced cardiac output during sinus rhythm. This is in agreement with Carleton and Graettinger, 1967. It may be referred to myocardial disease and ineffective atrial performance and in some patients the stenotic mitral valve may be the major factor.

According to Schroder, 1966, who used the same method as we did, quinidine in itself in the same dosage as was used in the present series did not lead to any significant difference in the hemodynamics in patients reclining and at rest. McIntosh and Morris, 1966 did not find that a plasma quinidine concentration of 3—4 mg/l affected either cardiac output or heart rate. Ferrer *et al.* who in 1948 observed a reduction in the systolic pressure after quinidine in patients at rest used a one time dose of quinidine. Their findings are thus not representative for the probable hemodynamic adaptation to quinidine during a continuous maintenance therapy.

Thus, there is no reason to assume that the observed changes during sinus rhythm in our series were caused by quinidine — on the contrary, they were caused by the normal rhythm.

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We have found that when there is a regular sinus rhythm at rest the heart rate usually becomes more normal and that the stroke volume increases and is the same from beat to beat At the same time the cardiac output increases in most cases and the peripheral vascular resistance decreases during sinus rhythm The fact that these conditions are applicable to patients in the supine position has been shown by many investigators cf review of literature, pp 13—15

Our results in patients at rest in a reclining position correspond well with the above-mentioned findings Furthermore, we were able to show that the diastolic pressure during sinus rhythm decreases and that the change in the heart rate on conversion of atrial fibrillation has a definite and linear relationship to the heart rate during atrial fibrillation Patients with a low ventricular rate during atrial fibrillation thus increase their rate while patients with a high rate decrease their rate with conversion to sinus rhythm A pronounced increase in the heart rate after conversion may lead to a simultaneous decrease in the stroke volume in spite of an increased cardiac output This could possibly explain the results of the investigation by Storstein and Tveten in 1955 cf p 13

Concerning the objective hemodynamic data, it is primarily during work load, that the superiority of sinus rhythm over atrial fibrillation appears First of all, the heart rate is adjusted in another manner since the sinus node has taken over the regulatory function Secondly the patient works with a higher cardiac output greater stroke volume and lower peripheral vascular resistance during sinus rhythm than during atrial fibrillation This applies both to patients with rheumatic heart disorder and to others Similar conditions have been described previously in patients during exercise in the supine position cf review of literature pp 13—15

These result are confirmed in our investigation with respect to exercise in a sitting position on a bicycle ergometer They are complemented by a finding of a lower diastolic pressure and a lower mean pressure in the brachial artery during sinus rhythm

Preliminary reports from the present series have been given by Varnauskas *et al* 1959 and Cramer *et al* 1960 No previous studies with several increased work loads in patients with atrial fibrillation and sitting on a bicycle ergometer seem to have been done in the same patients before conversion and during sinus rhythm After completion of the present series a similar study was published by Resnekov 1967

The change in the response of the heart rate to exercise is particularly remarkable in our series During atrial fibrillation there is a significantly non linear increase in the ventricular rate in relation to the intensity of the exercise with a sharp increase starting with a light work load After conversion there is a linear relation between the ventricular rate and the intensity of the exercise in the same patients just as in normal individuals

Furthermore the heart rate is lower during sinus rhythm than during atrial fibrillation even at the high work loads

This is in contrast to the findings of Killip and Baer, 1966 These authors showed that during moderate exercise in the supine position 24 hours after DC countershock conversion, there was a lower heart rate during sinus rhythm only in patients without valvular heart disease The influence of the DC countershock on the myocardium , exercise position or differences in the patient material or degree of digitalization may explain these differences

The heart rate response during atrial fibrillation cannot be prevented by digitalization, Wetherbee *et al*, 1952 A higher digitalis dose most likely will lead to a low heart rate at rest with extra systoles during exercise Conversion to sinus rhythm no doubt causes a better peripheral circulation adjustment to exercise than during atrial fibrillation

On the premise of the reduction in systolic pressure after a single dose of 0 8 g quinidine sulfate which was shown by Ferrer *et al*, 1948, it would be conceivable that quinidine caused the reduced peripheral vascular resistance after conversion to sinus rhythm This is not the case, however Quinidine causes no changes in the peripheral vascular resistance at the doses used after conversion, neither at rest nor during exercise, McIntosch *et al*, 1966, and Schroder, 1966

One question that has often been discussed is why the cardiac output increases during sinus rhythm compared to atrial fibrillation Two factors which may play a role are the more regular diastolic filling time during sinus rhythm, which causes an equal stroke volume, and the influence of the atrial contraction The latter factor, in particular, may play a greater role than has been previously assumed Sarnoff *et al*, 1962, Mitchell *et al*, 1962, and Braunwald and Frahm, 1961, have shown in dogs and humans, respectively, that a change in the relation between atrial contraction and ventricular contraction affects the latter and that the ideal situation is an atrial contraction which leaves the end diastolic pressure in the ventricle at an optimal condition for contraction The atrial contraction is also of importance for the closure of the mitral valve, Sarnoff *et al*, 1962

The results of the DC countershock seem to support the assumption that this also applies clinically Thus it happens frequently that there is no normal left atrial contraction in spite of electrical sinus rhythm until hours or days after the shock, Logan *et al*, 1965, Ikram *et al* 1967, 1968 This should correspond to the findings that the cardiac output does not always increase after a DC countershock, not until after a certain latency period, cf review of literature, p 14

The filling of the ventricle normally takes place primarily during the first part of the diastole and the atrial contraction then is probably of comparatively little significance for the filling of the ventricle On the other hand the final diastolic stretching of the musculature of the ventricle which is caused by an effective atrial contraction may be of importance to the energy developed especially in cases of hypertrophic left ventricle In cases of mitral stenosis, the ventricle is

not filled completely during the diastole and the atrial contraction plays a greater role with respect to the filling of the ventricle

Reduced risk of embolus during sinus rhythm

The risk of peripheral, arterial embolus is greatest in cases of combination of atrial fibrillation and mitral disease according to Askey, 1962 In our patient series all peripheral arterial emboli occurred during atrial fibrillation while there was no embolus during sinus rhythm during an individual follow up period of at least 28 months Thirty seven of 45 emboli occurred in patients with mitral disease This indicates that sinus rhythm protects against embolus This has been pointed out before but no comparable study has been published

Risks in connection with conversion

Another question which has frequently been discussed through the years is whether the risks connected with conversion from atrial fibrillation to sinus rhythm with quinidine are so great that they can be considered a contraindication to conversion attempts The complications observed at conversion have been acute attacks of syncope and the occurrence of emboli both in the pulmonary circulation and peripherally The latter risk may be disregarded as the results of several, large patient series (Maurice *et al* 1956 Blondeau *et al* 1962) using treatment with anticoagulants have shown that the risk of embolus is small In our patient series using anticoagulant treatment we did not have any case of arterial embolus in connection with the conversion attempt although we had an unselected material in which were included many patients with very large left atria and previous episodes of embolus

The risk of syncope at conversion with increasing quinidine doses is greater than the occurrence of embolus and in the present series much greater for women than for men Thus in our series we had 1[illegible] syncopal attacks none of which was fatal This particular point is of interest also in connection with DC countershock in regard to postconversion quinidine treatment

The results of the present study combined with the experience gathered in recent years permit the conclusion that patients have a better circulation during sinus rhythm than during atrial fibrillation at the same time that many patients improve subjectively The risk of peripheral arterial embolus is also markedly reduced during sinus rhythm The conversion procedure in itself nowadays, with DC countershock, does not constitute a pronounced risk for the patient although this is a matter of discussion, Resnekov and McDonald 1967

2 Selection of patients for conversion

With the background of these results and of previous experience one might say that conversion of atrial fibrillation is of advantage to the patient The

question then arises whether any particular patient group should be selected for, or excluded from, conversion attempts Age, diagnosis, duration of atrial fibrillation, subjective symptoms and the likelihood of maintaining sinus rhythm are factors which must be considered in connection with such a selection On these questions, our patient series gives certain information It is particularly easy to define clearly which patients should not be converted with quinidine or with DC countershock

Our patient series combined with the results of previous investigations — cf review of literature, pp 8—10 — show that a selection of suitable patients should be made prior to attempts to convert atrial fibrillation

For comparison it can be mentioned, that Radford and Evans, 1968, hold that the following patients ought to be converted from atrial fibrillation

1) Patients with treated thyrotoxicosis
2) Patients with atrial fibrillation without manifest heart disease especially if the arrhythmia is of a short duration
3) Patients with chronic rheumatic heart disease if the atrial fibrillation began after a cardiac operation.

In all other cases they found conversion of atrial fibrillation doubtful, especially if the duration of the fibrillation was more than 5 years

Lown, 1967, holds that the following patients are not suitable for cardioversion.

1) Patients who revert to atrial fibrillation in spite of adequate maintenance therapy of quinidine
2) Patients with mitral regurgitation and a giant left atrium, especially if the fibrillation is of more than 2 years duration
3) Elderly, asymptomatic patients with coronary artery disease
4) Patients with atrial fibrillation without manifest heart disease
5) Patients with paroxysmal atrial arrhythmias before atrial fibrillation
6) Patients with atrial fibrillation for more than 5 years
7) Patients immediately before or during valvular operation

According to our series the duration of the fibrillation is of great importance both for conversion frequency and for maintenance of sinus rhythm

It can definitely be said that patients with verified duration of fibrillation exceeding 3 years should not be subjected to conversion attempts either with quinidine or with DC countershock, regardless of the diagnosis The likelihood that such a patient will maintain his sinus rhythm is practically nonexistent This point has not been demonstrated clearly in previous investigations

On other hand, patients who have had atrial fibrillation for less than 6 months constitute a group in which the conversion results should be good. The likelihood that these patients will maintain sinus rhythm after the conversion is high. With respect to the high frequency of conversion, this has been shown clearly by Sokolow and Ball, 1956, Rokseth, 1963, and the present patient series

This applies regardless of whether the patient has RHD or not The follow up of our total patient series confirms this observation

The conversion frequency is particularly high in patients with RHD with a duration of fibrillation less than 6 months For durations of fibrillation longer than 6 months, regardless of whether the fibrillation has been present for only 6 months or for many years the conversion frequency is low It appears therefore to be important when a patient with RHD develops atrial fibrillation that one attempts to convert the arrhythmia without delay

When the duration of fibrillation exceeds 6 months, the cardiac size affects the conversion results in patients with RHD Patients with a roentgenological relative cardiac size exceeding 600 ml/m² BSA are converted less successfully It is therefore doubtful whether they should be subjected to conversion attempts with quinidine The literature does not show whether this is applicable also to DC countershock, but it appears likely

Sandoe 1967, was of the opinion that patients with operated mitral stenosis are not easier to convert than those who have not been operated upon In agreement with this observation is the finding in this patient series that there was no difference in the conversion results between patients with operated and not operated mitral stenosis On the other hand, there was a significantly better maintenance of sinus rhythm for ≧ 1 month in women with operated mitral stenosis than in women with non-operated mitral stenosis

For patients without RHD, the conversion frequency with quinidine is higher than for patients with RHD while the frequency of relapse to atrial fibrillation seems to be the same in both groups The first mentioned fact has previously been reported by among others Sandoe *et al* 196[illegible] who used a large unselected patient material

On [illegible] of our patient series we cannot [illegible] definite conclusions on the qu[illegible] whether or not one should [illegible] conversion attempts when there [illegible] fibrillation duration of between [illegible] months in patients without RHD Furthermore it is difficult to determine the fibrillation duration in this group It is probable however that the likelihood of the patient maintaining sinus rhythm decreases as the fibrillation duration increases

In the individual case the patient's status during atrial fibrillation — possibly also his response to exercise — will have to determine whether or not one should make a conversion attempt

Sex and age did not seem to be of importance with respect to conversion frequency or maintenance of sinus rhythm in our patient series It is particularly important to point out that the age of the patient does not affect the results Thus if there are other indications for conversion attempts relatively old patients can be converted This has also been shown in the results of several small patient series among others Frey, 1921 Viko *et al* 1923, and by Rokseth, 1963

The fact that the functional group in which the patients were placed before the conversion attempt affects the con-

version results was reported by Rokseth, 1963 In our patient series, we could not find any difference with respect to functional group, regardless of whether immediate conversion results or maintenance of sinus rhythm was involved

The hemodynamic conditions during atrial fibrillation at rest and the response to work load do not seem to affect the conversion frequency

The decisive importance of the fibrillation duration to the conversion results and to the maintenance of sinus rhythm might possibly be explained by the development of a degenerative functional or anatomical change in the sinus node, the conducting system as a whole or in the atrial myocardium during atrial fibrillation Progressive stretching of an atrial wall which does not contract could be one of the most important factors in this respect According to Frey, 1918[b], anatomic changes have been found in the sinus node during prolonged atrial fibrillation James, 1967, too, has recently shown that embolus or thrombosis in the sinus node artery may cause degenerative changes in the sinus node

Changes caused by the duration of fibrillation might also be reflected in the statistically higher concentrations of quinidine in plasma needed for conversion of patients with a duration of fibrillation exceeding 3 years in comparison with those with a shorter fibrillation

3 The importance of quinidine as maintenance therapy

This investigation did not include a comparison with matched controls to answer the question as to whether or not maintenance therapy with quinidine is of importance for the maintenance of sinus rhythm Such studies would be impossible to carry out One would need an enormous number of patients to find matched controls in a patient series as variable as the present one and as variable as the previously reported series

It was observed however, that in the patient group which contained 20 patients in whom the quinidine had to be discontinued altogether because of pronounced side effects, there was a significantly lower maintenance of sinus rhythm during the first month after the conversion than in the patient group which could take ≥ 1 2 g quinidine sulphate per day This dose was chosen as it was the highest dose that could be tolerated by most patients

Between the 2nd and the 28th month after the conversion, there was also a significantly better maintenance of sinus rhythm in the group which took ≥ 1 2 g quinidine sulphate per day than in the group which took a smaller maintenance dose or in whom quinidine was discontinued for one or another reason

Both of these findings indicate that it is important that the maintenance dose of quinidine be sufficiently large This applies both to the whole patient series and to patients with rheumatic heart disease In patients without rheumatic heart disease there was the same ten-

dency towards better maintenance of sinus rhythm with than without quinidine, but the differences were not significant

No statistical support for the importance of quinidine as a fibrillation prophylactic seems to have been published previously Information in the literature on the importance of quinidine as maintenance therapy is sparse in relation to the number of published reports which deal with the acute problems connected with conversion Sokolow and Ball 1956, reported that 1 6 g quinidine sulphate per day was the optimal maintenance dose and that without quinidine 85 per cent of the patients relapsed to atrial fibrillation within a week

Engstrom 1967, published a report on a series of converted patients with cardiosclerosis who had been converted by means of DC countershock Of these 29 patients had been treated with quinidine while 33 patients had not been given quinidine The standard dose was 1 6 g quinidine Durules® per day but the dose was reduced in some patients (the actual number was not given) Engstrom found no difference in the maintenance of sinus rhythm during a period of 3 months between these two groups It should be observed that the two patient groups were not quite comparable with respect to previous duration of the atrial fibrillation The group which was given quinidine included more patients (the actual number was not given) who had a fibrillation duration exceeding 6 months than the group to whom quinidine was not given

Hall and Wood, 1968, recently found no difference in maintenance of sinus rhythm with or without quinidine in 2 series of patients with RHD which they considered comparable The quinidine dose was 1 g daily The series were followed during a year

4 Determination of the quinidine concentration in plasma

The quinidine concentration in plasma was determined both during the conversion attempts and on repeated occasions in 12 patients during the follow up period The most important result of the latter part of the investigation was the great variability in the plasma concentration from patient to patient after the same quinidine dose During the follow up period, every patient had remarkably constant plasma values in relation to the dose of quinidine

The plasma concentration gives information which can be used as guidance in connection with the dosage of quinidine only when increasing quinidine doses are used during conversion attempts During the follow up period both after quinidine conversion and conversion by means of DC countershock the plasma concentration determination of quinidine is of importance only in cases in which the patient seems to tolerate the dose well but despite this reverts to atrial fibrillation A determination of the concentration under such

fying the duration of fibrillation in these patients

3 Sex, age, diagnosis with or without rheumatic heart disease, relative cardiac size or functional group did not influence the maintenance of sinus rhythm for one month or less than one month

There was a significantly better maintenance of sinus rhythm in men than in women after 4 years' observation

4 With a daily maintenance dose of quinidine $\geq$ 1 2 g quinidine sulphate sinus rhythm was maintained better during one month than in those patients in whom quinidine was withdrawn because of side effects This difference was significant for patients with rheumatic heart disease, as for the total patient series, while there was only a trend for patients without rheumatic heart disease Patients with a duration of fibrillation of more than 3 years were not included in these calculations

During the 2nd to 28th month after conversion sinus rhythm was significantly better maintained with a dose of > 1 2 g quinidine sulphate per day than on a smaller dose or no quinidine at all This applied to the whole patient series

5 A maintenance dose of quinidine Durules® gave an even quinidine concentration in plasma in the individual patient with a low coefficient of variation (< 25%) for the daily maximum concentrations

6 The following side effects of quinidine treatment were noted during the follow-up period in 148 converted patients gastrointestinal side effects in 61 patients (41 2%), which prompted discontinuation of quinidine in 13 patients, thrombocytopenia with increased bleeding tendency in 4 cases (2.7%) lowered platelet counts below 100 000/mm³ in 26 patients of 77 cases (34%) in whom platelet counts were done, urticaria with leucopenia in one patient (0 7%)

7 Increased atrio-ventricular conduction time (P R > 0 22 seconds) was found in 36 patients (24 4%) This was significantly more prevalent in patients with rheumatic heart disease than in those without rheumatic heart disease Arrhythmias other than atrial fibrillation, and for shorter periods than one week, were found in 30 patients (20 3%)

8 Forty-five peripheral emboli, all of which occurred during atrial fibrillation, were registered during the follow up period of at least 28 months in the total patient series Thirty-seven of these occurred in patients with mitral valvular lesions

9 Patients with atrial fibrillation and probably adequately treated with dicoumarol showed a lower frequency of emboli than patients without dicoumarol or on an insufficient dicoumarol dosage

10 Seventy-two of 237 patients died during the total observation time, i e 30 4% Of these, 27 died suddenly without signs of a cerebral embolus, 4 had maintained sinus rhythm and quinidine treatment, while 23 died in atrial fibrillation without quinidine All other patients died during atrial fibrillation without quinidine treatment

Comparisons between atrial fibrillation and sinus rhythm in the same patients

1 Relative roentgenological cardiac size with the patient in the prone position showed a significant increase with a mean of 17 ml immediately after conversion, and no difference 6—12 months later The change can not have any biological significance

2 ECG during exercise showed a significantly lower heart rate during sinus rhythm compared with atrial fibrillation

3 Hemodynamic investigations at rest in 24 cases before and after conversion showed significantly higher cardiac output lower heart rate, larger stroke volume, lower oxygen consumption lower diastolic pressure in the brachial artery and a lower peripheral vascular resistance in sinus rhythm than during atrial fibrillation The change in heart rate at conversion was dependent on the heart rate during fibrillation The rate increased or decreased so that a frequency of about 65 beats per minute was achieved

4 Hemodynamic investigations during work in 15 patients (200 kpm/min) showed a significantly increased cardiac output and a decreased heart rate during sinus rhythm concomitant with an increased stroke volume a decrease in mean brachial artery pressure and in peripheral vascular resistance

Statistically it could be shown in 7 patients that during atrial fibrillation the increase in heart rate was non linear with increasing work loads (200 and 400 kpm/min) During exercise in sinus rhythm in the same patients, the heart rate increased linearly with the work loads

Conclusions

1 Conversion from atrial fibrillation to sinus rhythm means a hemodynamic improvement for the patient

2 There is a decreased risk for peripheral arterial emboli during sinus rhythm compared with that during atrial fibrillation

3 Patients with a duration of atrial fibrillation of 3 years or more should not be subject to conversion as they do not maintain their sinus rhythm after conversion

4 Patients with rheumatic heart disease and atrial fibrillation for more than 6 months have a low conversion frequency with quinidine, if their cardiac size exceeds 600 ml/m² BSA

5 Patients with a duration of atrial fibrillation less than 6 months show a high conversion frequency, irrespective of the origin of their fibrillation and a better maintenance of sinus rhythm in patients with rheumatic heart disease than such patients with a longer duration of fibrillation

6 The frequency of peripheral arterial emboli during conversion with quinidine is very low provided that anticoagulants are administered

References

ASKEY, J. M.: Editorial. Embolism and atrial fibrillation. The effect of restoration of normal rhythm by quinidine. Amer. J. Cardiol. *9*, 491—495 (1962).

BARTLEY, O.: The isometric relaxation phase of the left ventricle. An electrokymographic study. Acta radiol. (Stockh.) Suppl. *198* (1960).

BECKWITH, J. R., IBARRA, J. A., and WOOD, J. E.: The problem of established atrial fibrillation. Amer. J. med. Sc. *231*, 519—530 (1956).

BENCHIMOL, A., ELLIS, J. G., DIMOND, E. G., and WU, T.: Hemodynamic consequences of atrial and ventricular arrhythmias in man. Amer. Heart J. *70*, 775—788 (1965).

BERLEY, B. S., and SALAND, G.: Febrile hypersensitivity to quinidine given orally. Report of two cases. J. Amer. med. Ass. *148*, 1121—1124 (1952).

BERMAN, R., and BLUMENTHAL, J. S.: Quinidine in auricular fibrillation. Minn. Med. *25*, 198—200 (1942).

BLONDEAU, M., COBLENTZ, B., and LENEGRE, J.: Incidents et accidents du traitement de la fibrillation auriculaire par la quinidine. Essai de prevention. Bull. Soc. med. Hop. Paris *76*, 631—637 (1960).

BLUMGART, H.: The reaction to exercise of the heart affected by auricular fibrillation. Heart *11*, 49—56 (1924).

BOIS, G.: An experimental study on serotonin (5-Hydroxytryptamine, 5HT) on man with special reference to renal function. Scand. J. clin. Lab. Invest. Suppl. 55 (1961).

BOLTON, F. G., and DAMESHEK, W.: Thrombocytopenic purpura due to quinidine. I. Clinical studies. Blood *11*, 527—546 (1956).

BRAUNWALD, E., and FRAHM, C. J.: Studies on Starling's law of the heart. IV. Observations on the hemodynamic functions of the left atrium in man. Circulation *24*, 633—642 (1961).

BROCH, O. J., and MULLER, O.: Haemodynamic studies during auricular fibrillation and after restoration of sinus rhythm. Brit. Heart J. *19*, 222—226 (1957).

BRODIE, B. B., and UDENFRIEND, S.: The estimation of quinine in human plasma with a note on the estimation of quinidine. J. Pharmacol. exp. Ther. *78*, 154—158 (1943).

BRODIE, B. B., UDENFRIEND, S., and BAER, J. E.: The estimation of basic organic compounds in biological material. I. General principles. J. biol. Chem. *168*, 299—309 (1947).

BRODIE, B. B., UDENFRIEND, S., DILL, W., and DOWNING, G.: The estimation of basic organic compounds in biological material. II. Estimation of fluorescent compounds. J. biol. Chem. *168*, 311—318 (1947).

CARLETON, R. A., and GRAETTINGER, J. S.: The hemodynamic role of the atria with and without mitral stenosis. Amer. J. Med. *42*, 532—538 (1967).

CONN, H. L., Jr.: Quinidine as an antiarrhythmic agent. Basic and clinical considerations. Advanc. cardiovasc. Dis. *2*, 286—304 (1964).

CRAMER, G., MALMCRONA, R., and VARNAUSKAS, E.: Haemodynamic effects at rest and during exercise of conversion of auricular fibrillation to sinus rhythm in patients with mitral valve disease. Acta tertii europ. de cordis scientia conventus Romae A. D. MDCCCLX, p. 261—263. Excerpta Medica Amsterdam (1960).

CRAMER, G., and ISAKSSON, B.: Quantitative determination of quinidine in plasma. Scand. J. clin. Lab. Invest. *15*, 553—556 (1963).

CRAMER, G., SANNERSTEDT, R., TIBBLIN, G., and WERKO, L.: Hjartarytmer och kinidin, p. 40—53. AB Hassle Goteborg (1963).

CRAMER, G., VARNAUSKAS, E., and WERKO, L.: A new quinidine preparation with sustained release. Acta med. scand. *173*, 511—519 (1963).

CRAMER, G.: Kinidinkoncentration i plasma — kinidinsulfat contra kinidinduretter. In Hjartarytmer p. 183—189. Goteborgs symposier 1963. Edited by E. Varnauskas, R. Sannerstedt & L. Werko. AB Hassle, Goteborg (1964).

CULLHED, I., HOLMDAHL, M. H., SON and MALERS, E.: Extern elektrostötschock vid supraventrikulära arytmier. Svenska Läk.-Tidn. *61*, 742—757 (1964).

DAHLBERG, G.: Statistical methods for medical and biological students. George Allen and Unwin, London (1948).

DAVIES, P., LEAK, D., and ORAM, S.: Quinidine induced syncope. Brit. med. J. II, 517—520 (1965).

DIPALMA, J. R., and SCHULTZ, J. L.: Antifibrillatory drugs. Medicine *29*, 123—168 (1950).

EDGAR, A. L., and SOKOLOW, M.: Experiences with the photofluorometric determination of quinidine in blood. J. Lab. clin. Med. *36*, 478—484 (1950).

ELLIS, A. W. M., and CLARK-KENNEDY, A. E.: Arrest of auricular fibrillation by use of quinidine. Lancet II, 894—898 (1921).

ENGSTROM K Kinidinprofylax efter regulariseriug av formaksflimmer The value of quinidine in the prevention of atrial fibrillation after electrical conversion Opusc. med (Stockh) *12* 24—27 1967

FERRER, I HARVEY R. M WERKO L DRESDALE D T COURNAND A and RICHARDS D W Jr Some effects of quinidine sulfate on the heart and circulation in man Amer Heart. J *36* 816—837 1948

FERRER, I M and HARVEY R M The value of converting atrial fibrillation to normal sinus rhythm Amer Heart J *68* 725—727 1964

FISCHERMANN K and SCHLEISNER P Atrieflimmen hos patienter over 50 ar behandlet med kinidin Nord Med *43* 705—706 1950

FORSBERG S Å Pulmonary blood volume in man Acta med scand Suppl 410 1964

FREEDMAN A L BARR P S and BRODY E A Hemolytic anemia due to quinidine observations on its mechanisms Amer J Med *20* 806—816 1956

FREEMAN I and WEXLER J Quinidine in chronic atrial fibrillation Amer J med Sci *239* 181—186 1960

FREEMAN I and WEXLER J Atrial fibrillation anticoagulation and quinidinization Sth med J (Bgham Ala) 60 13—17 1967

FREY W Über Vorhofflimmern beim Menschen und seine Beseitigung durch Chinidin Berl klin Wschr *55* 450—452, 1918

FREY W Weitere Erfahrungen mit Chinidin bei absoluter Herzunregelmaßigkeit Berl klin Wschr *55* 849—853 1918b

FREY W Chinidin zur Bekampfung der absoluten Herzunregelmaßigkeit (Vorhofflimmern) Dtsch Arch. klin Med *136* 70—87 1921

FRIEDBERG C. K Diseases of the heart p 549 3rd ed W B Saunders Philadelphia and London 1966

FRIEDBERG R and SJOESTROEM B Quinidine treatment of auricular fibrillation Acta med scand *155* 293—305 1956

GERSTENBLITH T KATABI G STEIN I and TIRSCH H. Quinidine utilization in cardiac arrhythmias Report of study involving sulfate and polygalacturonate N Y St J Med *66* 701—706 1966

GILBERT R AUCHINCLOSS J H Jr EICH R SMULYAN H and KEIGHLEY J Exercise performance before and after conversion from atrial fibrillation to sinus rhythm Clin Res 9 139 1961

GOLDMAN M J Quinidine therapy of auricular fibrillation Amer Heart J *40* 93—99 1950

GOLDMAN M J Quinidine treatment of auricular fibrillation Amer J med Sci *222* 382—391 1951

GOLDMAN M J The management of chronic atrial fibrillation. Indications for and method of conversion to sinus rhythm Progr cardiovasc Dis *2* 465—479 1959

GOODMAN L. S and GILMAN H The pharmacological basis of therapeutics 3rd ed p 705—706 Mac Millan New York 1965

GRAETTINGER J S CARLETON R A and MUENSTER J J Circulatory consequences of changes in cardiac rhythm produced in patients by transthoracic directcurrent shock J clin Invest *42* 938—939 1963

GRAETTINGER J S CARLETON R A and MUENSTER J J Circulatory consequences of changes in cardiac rhythm produced in patients by transthoracic directcurrent shock J clin Invest *43* 2290—2302, 1964

HALL H Beitrag zur Behandlung des Vorhofsflimmerns Zusammenfassung Acta med scand *173* 162—163 1941

HALL J I and WOOD D R Factors affecting cardioversion of atrial arrhythmias with special reference to quinidine Brit Heart J *30* 84—90 1968

HALMOS P B The prognosis of atrial fibrillation after mitral valvotomy Amer Heart J *72* 30——34 1966

HAMFELT A and MALERS E Determination of quinidine concentration in serum in the control of quinidine therapy Acta Soc Med upsalien *68* 181—191 1963

HANSEN W R McCLENDON R L and KINSMAN J M Auricular fibrillation Hemodynamic studies before and after conversion with quinidine Amer Heart J *44* 499—516 1952

HAY J The action of quinidine in the treatment of heart disease Based on the experiences of certain members of the Cardiac Club Lancet II 543—545 1924

HECHT H H OSHER W J and SAMUELS A J Cardiovascular adjustments in subjects with organic heart disease before and after conversion of atrial fibrillation to normal sinus rhythm J clin Invest *30* 647—648 1951

HERDAN G Statistics of therapeutic trials 1st ed Elsevier Amsterdam 1955

HEWLETT A W and SWEENEY J P The quinidine treatment of auricular fibrillation J Amer med Ass *77* 1793—1797 1921

HOLZMAN D and BROWN M. G The use of quinidine in established auricular fibrillation and flutter Amer J med Sci *222* 644—652, 1951

HURST J W PAULK E. A PROCTOR H. D and SCHLANT R C Management of patients with atrial fibrillation Amer J Med *37* 728—741 1964

HYRENIUS H Statistiska metoder 4th ed Almqvist & Wicksell Stockholm 1962

IKRAM H NIXON P G F and ARCAN T Atrial function following cardioversion Amer Heart J *74* 729—730 1967

IKRAM H NIXON P G F and ARCAN T Left atrial function after electrical conversion to sinus rhythm Brit Heart J *30* 80—83 1968

JAMES T N Pathology of small coronary arteries *Amer J Cardiol 20 679—691 1967*

JONSELL, S A method for the determination of the heart size by teleroentgenography (A heart volume index) Acta radiol. (Stockh.) *20* 325—340 1939

KERKHOF A. C. *and* BAUMANN H Minute volume determinations in mitral stenosis during auricular fibrillation and when restored to normal rhythm. Proc. Soc. exp Biol. *31 168—170 1933/34*

KILLIP T. Synchronized DC precordial shock for arrhythmias. Safe new technique to establish normal rhythm may be utilized on an elective or an emergency basis J *Amer med.* Ass *186* 1—7 1963

KILLIP T and BAER, R A Hemodynamic effects after reversion from atrial fibrillation to sinus *rhythm by precordial shock J clin Invest. 45* 658—671 1966

KISSANE, R. W CONN J J and ROSE, S M Atrial fibrillation. Reversion to normal sinus rhythm *Dis Chest 39* 299—304 1961

KJELLBERG S R. RUDHE, U and SJOSTRAND T The relation of the cardiac volume to the weight and surface area of the body the blood volume and the physical capacity for work Acta radiol. (Stockh.) *31* 113—122 1949

KJELLBERG S R. MANNHEIMER, E. RUDHE, U and JONSSON B. Diagnosis of congenital heart disease 2nd ed p 124—130 Year Book Publishers Chicago 1959

KORSGREN M. LESKINEN E. PETERHOFF V BRADLEY E. and VARNAUSKAS E. Conversion of atrial arrhythmias with DC shock. Primary results and a follow up investigation. Acta med scand. Suppl 431 1965

KORY R. C. and MENEELY G R. Cardiac output in auricular fibrillation with observations on the effects of conversion to normal sinus rhythm. J clin. Invest *30* 653 1951

KUTTI J and WEINFELD A. The frequency of thrombocytopenia in patients with heart disease treated with diuretics Acta med. scand. In press 1968

LAAKE, H Erfaringer med kinidinbehandling ved aurikulaer flimmer Nord. Med. *26 820—824* 1945

LARSSON H. and KJELLBERG S R Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol. (Stockh) *29* 159—177 1948

LARSSON R. K. The mechanism of quinidine purpura *Blood 8* 16—25 *1953*

LEHMANN J Protrombinbestamning i kliniken. En ny modifikation av Quicks metod. Nord. Med. *12* 3192—3197 1942.

LEMBERG L CASTELLANOS A SWENSSON J and GOSSELIN A Arrhythmias related to cardioversion. Circulation *30* 163—170 1964

LEVY R L. Clinical studies of quinidine. IV The clinical toxicology of quinidine J Amer med Ass *79 1108—1113* 1922.

LOGAN W F W E ROWLANDS D J HOWITT G and HOLMES A M. Left atrial activity following cardioversion. Lancet II 471—473 1965

LOWN B Cardioversion. New Engl. J Med. *269* 1101 1963

LOWN B Electrical reversion of cardiac arrhythmias Brit. Heart J *29* 469—489 1967

LOWN B AMARASINGHAM R and NEUMAN J. New method for terminating cardiac arrhythmias Use of synchronized capacitator discharge. J Amer med. Ass *182* 548—555 1962a

LOWN B NEUMAN J AMARASINGHAM R and BERKOVITS B V Comparison of alternating current with direct current electroshock across the closed chest. Amer J Cardiol *10* 223—233 1962b

LOWN B PERLROTH M. G KAIDBEY S ABE, T and HARKEN D E. Cardioversion of atrial fibrillation. A report on the treatment of 65 episodes in 50 patients New Engl. J Med *269* 325—331 1963

LYON A F and DEGRAFF A. C. Antiarrhythmic drugs Part III Quinidine toxicity Amer Heart J *70* 139—141 1965

MCDONALD L. RESNEKOV L and O BRIAN K Direct-current shock in treatment of drug resistant cardiac arrhythmias Brit. med J 1 1468 —1470 1964

MCINTOSH H D and MORRIS J J. The hemodynamic consequences of arrhythmias Progr cardiovasc. Dis *8* 330—363 *1966*

MCMILLAN R. L. and WELFARE, C. R. Chronic *auricular fibrillation Its treatment with quinidine* sulphate J *Amer* med. Ass *135* 1132—1136 1947

MALMCRONA R. Haemodynamics in myocardial infarction. Acta med. scand. Suppl. 417 1964

MAURICE, P ACAR J RULLIERE, R and LENEGRE J Traitement par la quinidine de 390 cas de fibrillation auriculaire. Arch Mal. Coeur *49* 615—636 1956

MAYNARD E. P Five years experience in the treatment of chronic auricular fibrillation with quinidine sulphate Amer J med Sci *175* 55—60 1928

MILLER H S Synchronized precordial electroshock for control of cardiac arrhythmias Initial results and success after three months J Amer med Ass *189* 549—552, 1964

MITCHELL, J H. GILMORE, J P and SARNOFF S J The transport function of the atrium Factors influencing the relation between mean left atrial pressure and left ventricular and diastolic pressure Amer J Cardiol *9* 237—247 1962.

MORRIS J J Jr KONG Y NORTH W C. and MCINTOSH H D Experience with "Cardioversion" of atrial fibrillation and flutter Amer J Cardiol. *14* 94—100 1964

MORRIS J J Jr ENTMAN M NORTH W C KONG Y and McINTOSH H D The changes in cardiac output w th revers on of atrial fibrilla tion to s nus rhythm Circulation *31* 6 0—6 8 1965

MOSBECH J and FROLUND THOMSEN V K n d n behandling af atr eflimren Ugeskr Laeg *121* 49—54 1959

Nomenclature and criter a for d agnos s of the diseases of the heart and blood vessels 5th ed p 80—81 New York Heart Ass Inc New York 1953

ORAM S DAVIES J P H WEINBREN I TAGGART P and KITC IEN L D Convers on of atrial fibr llation to s nus rhythm by d rect-current shock Lancet II 159—162 1963

ORAM S and DAVIES J P H Further exper ence of electr cal convers on of atr al fibrillat on to sinus rhythm Analys s of 100 pat ents Lancet I 1294—1298 1964

OSHER H L. and AUSTIN W H Thrombocyto pen c purpura due to quin dine J Ma ne med Ass *54* 199—202 1963

PARKINSON J and CAMPBELL, M The qu nid ne treatment of aur cular fibr llation Quart J Med *22* 281—303 1929

PAULIN S and VARNAUSKAS E Select ve trans septal ang ocardiography Acta rad ol (Stockh) *57* 3—10 1962

PEDERSEN A and LARSEN O A Cardial arrhyth m as treated w th synchronized DC defibrilla t on Acta med scand. *179* 59— 0 1966

RADFORD M D and EVANS D W Long term results of DC revers on of atrial fibrillation Br t Heart J *30* 91—96 1968

RESNEKOV L. Haemodynamic studies before and after electr cal convers on of atr al fibr llat on and flutter to sinus rhythm Br t Heart J *29* 700—08 1967

RESNEKOV L and McDONALD L Complications in 220 patients w th card ac dysrhythmias treated by phased direct current shock and ind cat ons for electro-convers on Br t Heart J *29* 926—936 1967

RODMAN T PASTOR B H and FIGUEROA W Effect on cardiac output of convers on from atr al fibr llation to normal sinus mechan sm Amer J Med *41* 249—258 1966

ROKSETH R Clin cal cons derat ons n qu nid ne therapy of chron c aur cular fibrillation A study of 200 unselected pat ents — w th follow up Acta med scand *1 4* 171 1 8 1963

ROKSETH R and STORSTEIN O Quin dine therapy of chron c aur cular fibrillation. The occurrence and mechanism of syncope Arch ntern Med *111* 184—189 1963

ROSE, O A VOGL A and TURTZ A I Drug fever due to quinidine New Engl. J Med *248* 96—98 1953

STRATFORD E. C. and TANAKA K. R. Quinidine-induced thrombocytopenic purpura. Calif Med. *102* 310—313 1965

STURNICK, M. L. An unusual toxic manifestation of the oral use of quinidine sulfate. Amer Heart J *24* 559—561, 1942.

THOMSEN G. Quinidine as a cause of sudden death. Circulation *14* 757—765 1956

UDENFRIEND S. Fluorescence assay in biology and medicine = Molecular Biology 3 401—403 1962 Academic Press New York 1962.

VARNAUSKAS E. CRAMÉR G. MALMCRO A, R. DAHL, L-E. NYSTROM B. WASSEN A. and WERKÖ L. Regularisering av atrieflimmer vid mitralisvitium. Nord. Med. *62* 1109—1114 1959

VIKO L. E. MARVIN H. M. and WHITE, P. D. A clinical report on the use of quinidine sulphate. Arch intern Med *31* 345—363 1923

WASSEN A. The use of bromsulphalein for determination of the cardiac output. Scand J clin Lab Invest. *8* 189—192, 1956

WEISMAN S. Reminders on the use of quinidine. Geriatrics *21* 186—188 1966

WENCKEBACH K. F. Die unregelmaßige Herztatigkeit und ihre klinische Bedeutung. p. 125, Engelmann Leipzig & Berlin 1914

WETHERBEE D. G. BROWN M. G. and HOLZMAN D. Ventricular rate response following exercise during auricular fibrillation and after conversion to normal sinus rhythm. Amer J med Sci *223* 667—670 1952.

WOLFF L. and WHITE, P. D. Auricular fibrillation. *Results of seven years experience with quinidine sulphate therapy (1921—1928).* Arch intern Med. *43* 653—675, 1929

YOUNT E. H. ROSENBLUM M. and MCMILLAN R. L. Use of quinidine in treatment of chronic auricular fibrillation. Arch intern. Med *89* 63—69 1952